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14. ABSTRACT

Feral swine (*Sus scrofa domesticus*) are spreading across North America at an alarming rate. Four Canadian provinces and 39 states within the continental United States now report standing populations of feral pigs. Estimates place the number of feral hogs in Texas >2M, accounting for more than half of the U.S. population. It is known that feral swine impact local ecology following establishment, with regard to shifts in local flora and fauna. The overall objective of this research was to investigate the role of feral swine in tick-borne pathogen transmission in Texas. The underpinning objectives were to establish host records for tick species parasitizing feral swine, determine the species assemblages associated with feral swine among different ecoregions of Texas, determine by immunoassay to which tick-borne bacteria feral pigs were being exposed, and detect the DNA of tick-borne bacteria by polymerase chain reaction (PCR) assay in the event of poor or early immune response by the host. Feral pigs (N=432) were harvested from June 2008 to June 2010 using box and corral traps and by aerial gunning. Seven species of ticks, *Amblyomma americanum*, *A. cajennense*, *A. maculatum*, *Dermacentor albipictus*, *D. halli*, and *D. variabilis*; and *Ixodes scapularis*, were collected. Immature stages of *A. cajennense* and *A. americanum* were collected as well. All classes of feral pigs, gender by age, were infested with ticks. Serum was collected through a multi-organizational effort from 2006 to 2010 and tested by ELISA for previous exposure to tick-borne pathogens in the genera *Rickettsia* and *Ehrlichia* (N=888) and *Borrelia* (N=849). Prevalence percentages by immunoassay were 27.59%, 13.18%, and 2.12% for *Rickettsia*, *Ehrlichia*, and *Borrelia*, respectively. Samples positive by ELISA for exposure to *Borrelia* were further screened by Western Blot for exposure to *Borrelia turicatae*. The results were equivocal in most cases. Blood samples (N=233) were collected from 2008 to 2010 and analyzed by PCR for the detection of the DNA of these same three genera of bacteria. Two of the samples were positive by PCR for the presence of *Borrelia* DNA. Texas feral swine are serving as hosts for at least seven species of ticks and are interacting with tick-borne pathogen transmissions cycles in Texas.

15. SUBJECT TERMS
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TICKS AND TICK-BORNE PATHOGENS ASSOCIATED WITH FERAL SWINE IN EDWARDS PLATEAU AND GULF PRAIRIES AND MARSHES ECOREGIONS OF TEXAS

A Dissertation

by

DAVID M. SANDERS

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2011

Major Subject: Entomology

Ticks and Tick-Borne Pathogens Associated with Feral Swine in Edwards Plateau and Gulf Prairies and Marshes Ecoregions of Texas

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Approved by:

Co-Chairs of Committee,	Pete D. Teel
	Albert Mulenga
Committee Members,	Don S. Davis
	Ian R. Tizard
	Johnathan L. Kiel
Head of Department,	David W. Ragsdale

May 2011

Major Subject: Entomology

ABSTRACT

Ticks and Tick-Borne Pathogens Associated with Feral Swine in Edwards Plateau and Gulf Prairies and Marshes Ecoregions of Texas. (May 2011)

David M. Sanders, B.S., University of Memphis; M.S., University of Tennessee

Co-Chairs of Advisory Committee: Dr. Pete D. Teel
Dr. Albert Mulenga

Feral swine (*Sus scrofa domesticus*) are spreading across North America at an alarming rate. Four Canadian provinces and 39 states within the continental United States now report standing populations of feral pigs. Estimates place the number of feral hogs in Texas >2M, accounting for more than half of the United States population. It is known that feral swine impact local ecology following establishment, with regard to shifts in local flora and fauna.

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Serum was collected through a multi-organizational effort from 2006 to 2010 and tested by ELISA for previous exposure to tick-borne pathogens in the genera *Rickettsia* and *Ehrlichia* (N=888) and *Borrelia* (N=849). Prevalence percentages by immunoassay were 27.59%, 13.18% and 2.12% for *Rickettsia*, *Ehrlichia*, and *Borrelia*, respectively. Samples positive by ELISA for exposure to *Borrelia* were further screened by Western Blot for exposure to *Borrelia turicatae*. The results were equivocal in most cases. Blood samples (N=233) were collected from 2008 to 2010 and analyzed by polymerase chain reaction for the detection of the DNA of these same three genera of bacteria. Two of the samples were positive by PCR for the presence of *Borrelia* DNA.

Texas feral swine are serving as hosts for at least seven species of ticks and are interacting with tick-borne pathogen transmissions cycles in Texas.

DEDICATION

This manuscript is dedicated to small road signs at major crossroads.

Chattanooga, TN-The realization human terms are horribly finite. Thank you!

New York City -"If you think you can fit through there then have at it!" Linda Sanders proving sometimes bullies, or New York cabbies, have to be dealt with on their level.

Milan, TN and Ft. Hood, TX-Thank you DB and DC, respectively, for helping me learn how to follow mom's example, and you both are most welcome for the lessons.

Illesheim, Germany-"My son is!" Donald M. Sanders' response to "Who's your best third baseman?"

West Ft. Hood, TX-"David, you can have as many jobs as you want in life as long as you make a contribution at each one!" Retired USAF colonel

El Paso, TX-"Do something you love and you'll never work a day in your life." Radio commentator Paul Harvey

Memphis, TN-"I do not see you counting pills the rest of your life. I think you should take the GRE and see how you do . . ." Dr. James F. Payne

Brooks Air Force Base, San Antonio, TX-". . . I didn't get your packet for this PhD funding . . ." Col James Swaby

El Paso, TX-Nancy Burbank's comment is one that I won't repeat here, but it was made to Christine (Burbank) Sanders during a mother-daughter discussion about Christine's worthless new boyfriend. Thanks Nan for the proper motivation to ask before she wised up.

El Paso, TX-"Yes" Christine (Tina) Burbank . . . Sanders

Donald, Kellen, Caleb and Aidan, I'm as proud of you as a father could be, and every second I spend with each of you is a new directional arrow. Thank you for putting up with an imperfect and absent dad.

ACKNOWLEDGEMENTS

Every personal accomplishment requires help, guidance and/or tolerance of those we encounter along the road to the desired end. Proper committee selection is crucial to the success of any higher degree pursuit. But, for those of us in the United States Armed Forces, proper committee selection not only defines the outcome of the project, it also defines our careers. With that said, I owe a tremendous debt to my committee, starting with my co-chair Dr. Pete D. Teel. Dr. Teel was always enthusiastic about my work and slow to dismiss something because it was not working at the time. Chapter IV exists because Dr. Teel saw the relevance in countless hours of work to which I was blinded by continuous frustration. Dr. Albert Mulenga, also a co-chair, was what one expects to find in a research university, daring. I truly appreciate his "let's try this" approach. Any idea was okay as long as it was well-founded. Science should have more like him. I also appreciate his tolerance of his missing-in-action graduate student. Dr. Mulenga never showed frustration towards my lab hopping to get my work done. Drs. Teel and Mulenga, my departmental committee members, kept the project focused on medical entomology.

Drs. Don Davis, Ian Tizard, and Johnathan Kiel provided the novelty that doctoral projects are supposed to have. "Dr. Davis is great to work with!" was a comment that I heard from everyone I queried prior to asking Dr. Davis to be a committee member. The comment did not do him justice. Dr. Davis provided much needed reality checks, allowed me to tag along on projects solely for my edification, and Dr. Davis knew that good advice was often better delivered "off-site". Dr. Tizard was one of those people that I knew I would be putting a lot on the line if I asked him to serve on my committee, but taking his class showed me that I would be remiss in not having him on my committee. When he asked the other committee members and me, "What about this course work . . . When is it done?" He meant it was time to get to work. I

knew right then I had made the right decision. Dr. Kiel provided everything from scientific prospective to financial support to the best and most relevant question I was asked during my program. His question was, paraphrasing, "did you have convergence . . ."

Just behind a good committee is the need for a quality collaborative network. My network ranged from infectious agents labs to federal research labs to boots on the ground. I could not have asked for better. The project would never have gotten off the ground had it not been for permission agreements on the part of Dr. Lynn Drawe with the Welder Wildlife Refuge and Mr. Lucas Cooksey with Camp Bullis Military Reservation's environmental office. Dr. Drawe retired shortly after the project began, and Dr. Terry Blakenship picked up where Dr. Drawe had left off. Terry and Lucas took site permission to a whole new level. Both were active in trap site selection, pre-baiting, and processing animals. They both went well past the point of helpful.

One of the first additional considerations Terry showed me was when he coordinated my work with that of Dr. Tyler Campbell, USDA-APHIS-WS-National Wildlife Research Center's Kingsville, TX field station leader. Half of the samples taken from Welder Wildlife Refuge were taken thanks to Tyler and his assistant, Mr. David Long. Their help made the Welder Wildlife Refuge part of my project what it is.

The boots on the ground around the rest of Texas was largely accomplished by the personnel of the Texas Wildlife Service Program. This is a cooperative group made up of USDA-APHIS-Wildlife Services and Texas Agrilife Wildlife Services. Most of these folks I have never met, but they took the time out of their day to assist me with my project just for the asking. There were others though who I did interact with personally, and they bear mentioning by name. Mr. Gary McEwen, Mr. Doug Steen and Janean Romies were helpful to a fault. Janean sent literally hundreds of samples from over most of Texas and asked only that her group

be acknowledged. Gary dragged me along, going out of his way to get permission, and never asked for a thing in return. Doug set traps and collected samples as an additional duty in his day and never asked for a thing in return. All of the supervisors and local government trappers have my sincerest thanks.

Committee selection, site agreements and collaborators in the field was the foundation of my project, but there was still the need for funding, sources of materials and organisms, and area expertise. Funding assistance, though not always monetary, came largely from the Air Force Research Laboratory (AFRL) out of San Antonio, TX and Wright-Patterson Air Force Base, Dayton, Ohio. The individuals largely responsible were Dr. Johnathan Kiel, Dr. Richard Stotts, Mr. Mark Fagan, Major Keith Blount and Captain Wesley Walker. I was also funded by the Armed Forces Pest Management Board. Lieutenant Colonel Doug Burkett was instrumental in finding the correct avenues through which to connect me with Defense Warfighter Protection Program funds.

Drs. David H. Walker, Donald H. Bouyer, and Tom G. Schwan made the serology and DNA detection possible. Dr. Bouyer supplied me with the *Rickettsia rickettsii* and *Ehrlichia chaffeensis* used as the antigen source in the ELISA assay and the DNA template for controls in the PCR assays. Dr. Schwan supplied the *Borrelia turicatae* used for the same purposes. However, Dr. Schwan went even further in opening his lab to me. I was able to complete the serology portion only because he and his lab personnel, Dr. Job Lopez, Ms. Sandra Stewart and Ms. Brandi Williamson, devoted a week of long days to my project without any request for return of investment. I owe the bulk of the success with my project to the folks mentioned in this paragraph. Their kindness made two of the chapters possible.

Then there is the “all others” group, but they are definitely the “last but not least” group as well! These are the folks that put up with me on a daily basis and have seen my jaw clinched,

heard utterances that could peel paint, broken bread with me, and stuck tight despite it all. Mr. Otto Strey is at the top of the list. He definitely fits into all categories plus the putting up with the thousands of fleas, which alone would test the mettle of most! Major Anthony Schuster is another who has also put up with freezing cold and blistering heat just so we could pull the ticks, fleas and lice off of feral pigs, but the purchase trip he allowed me to tag along on is one my son, Caleb, will always remember thanks to a wore out single-shot Remington .22 rifle. Dr. Pam Ferro was the most meddling individual I knew during my time at Texas A&M University. Thank you, Pam! Kellen, Caleb and Aidan, 2007- 2011 was rough on us all, but I hope there were at least some enjoyable moments for you, there certainly were for me despite the fleas.

I didn't just appear at Texas A&M University one day. This took years and countless kind and helpful people to get me here. I met Drs. James Payne and Jack W. Grubaugh at the University of Memphis, and they both left an indelible mark on the way I perceive science and life today. The same holds true for Drs. Carl Jones, Arnold Saxton and Reid Gerhardt at the University of Tennessee. How I view my own language, problem solving and the world of science was changed by these three men. Then there were those who put their name on the line by writing letters on my behalf. Col Sean Scully, Col Tom Duquette, and Dr. Richard Stotts were willing to risk their own reputations so that I could pursue a doctorate with no possible personal return except to show me kindness.

There is always the mention of family in acknowledgements, and I will definitely do so as well. My thanks go to my father and mother for teaching me about faith and God. They also taught me how to work hard and how to keep on keeping on, perseverance! But, the blessings that are my family, Tina, Kellen, Caleb, Aidan and especially Donald, have tolerated my absences for years, and I thank them for always welcoming me back. I also give them my word that this was the last time, save for service to my country and my Maker!

NOMENCLATURE

CBMR	Camp Bullis Military Reservation
ELISA	Enzyme Linked Immunosorbent Assay
LBRF	Louse-Borne Relapsing Fever
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
TCB	Texas Canine Borrelia Cases
TRL	Tick Research Laboratory
TBRF	Tick-Borne Relapsing Fever
WWR	Welder Wildlife Refuge

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER I

INTRODUCTION

The role of biodiversity in increased disease transmission has been reviewed extensively in recent years (Ostfeld 2009, Kessing et al. 2010). Osfeld (2009) made the argument that high biodiversity can buffer against disease transmission by reducing reservoirs, reducing encounter rates among vectors and reservoirs or among reservoirs. This is based on the prevailing working hypothesis that dilution occurs in areas of increased biodiversity (Ostfeld and Keesing 2000a, Ostfeld and Keesing 2000b).

The more accurate hypothesis is that the issue lies with biodiversity shifts that favor transmission such as introduction of a new pathogen onto a landscape inhabited by naïve populations e.g. West Nile Virus' introduction to the United States. West Nile Virus was an addition to the diversity of mosquito-borne pathogens endemic to the United States. Ostfeld (2009) argues that West Nile Virus transmission to humans is reduced in areas of greater avian species diversity. However, it could be argued that transmission of West Nile Virus in the United States would not exist had biodiversity among mosquito-borne viruses not increased. Ostfeld (2009) deviates from overall biodiversity slightly when addressing Lyme Borreliosis in the northeastern United States by narrowing the hypothesis to the hosts of the tick vector *Ixodes scapularis* and hosts with reservoir competency, not so much on overall species diversity. The increase in transmission due to decreased biodiversity can obviously be debated and garners attention versus reduced biodiversity where no transmission occurs or when diversity increases and there are no detectable adverse or objectionable outcomes.

This dissertation follows the style of the Journal of Vector Ecology.

A decrease in pathogen transmission resulting from the proposed dilution hypothesis can only work, in the case of vector-borne pathogen transmission, if the increased biodiversity is among the vectors' host populations of competent reservoirs and susceptible hosts. This requires that the pathogen is not so pathogenic as to cause high mortality in the alternate host and no alternate modes of transmission. An increase in competent vector species would increase the opportunity for pathogen transmission, all other factors withstanding. If the diversity among viable host and competent vector species increased, pathogen transmission should remain the same percentage wise, again all other factors withstanding, by a corresponding increase in all vectors and all hosts in the system.

Aedes aegypti, an old world mosquito species, is a known competent vector of Yellow Fever Virus, is highly peridomestic and was previously widely distributed across the eastern half of the United States. *Aedes albipictus,* now firmly established across the United States following its inadvertent introduction to the United States in the late 1980s, has been shown in the laboratory to be a competent vector of several organisms considered to be pathogenic to humans (Juiliano and Lounibos 2005). It would appear that disease transmission of mosquito-borne pathogens should have risen with the arrival of a second competent vector. However, it turns out that *Aedes albipictus* displaces *Aedes aegypti* and in fact influenced the virtual eradication of Yellow Fever in the United States (Juilano and Lounibos 2005). Granted there were other factors that influenced Yellow Fever transmission in the United States. But, Yellow Fever increased with the introduction of *Aedes aegypti* and a susceptible host population. This shift in biodiversity, increase, favored transmission. Yellow Fever cases in the United States decreased following abatement efforts and the accidental introduction of the interspecific competitor *Aedes albipictu*. Unfortunately, transmission of pathogenic organisms, especially vector-borne

organisms, is seldom so straight forward as this implies, and the situation is further complicated by today's global societies and economies.

A major issue arising from globalization is the rise of invasive species. Crowl et al. (2008) reviewed the effects of invasive species and infectious diseases on localized ecology, and they submit the view that invasive species and infectious diseases are becoming more prevalent. Parker et al. (1999) address shifts (reductions) in richness and abundance while examining the impact of invasive species on native communities. Pimental et al. (2005) went further by developing cost estimates for the more problematic invasive species. The species of interest here is the feral pig (*Sus scrofa domesticus*, *Sus scrofa scrofa* and their crosses). Pimental et al. (2005) estimated the annual cost of damage caused to native and managed landscapes by feral pigs at $800M per year for the United States., and they suggested the 2005 population of feral pigs in Texas between 1-1.5M individuals with a daily cost from damage of $1K per pig. Burns (2007) placed the Texas population at 2M, approximately a two-fold increase in two years. They also noted the cost associated with trying to control feral pig populations by citing the cost of control of feral pigs in state parks of Hawaii. They estimated the cost for three parks to be $450K annually in 2005. Kotanen (1995) showed that feral pigs significantly alter plant life in areas of the California coastal prairies where feral pigs grubbed for food. Coblentz and Baber (1987) had different results in their study area of Isla Santiago, Galapagos, Ecuador. Coblentz and Baber attributed this to the fact that the vegetation community had evolved under heavy use of the native giant tortoise. However, Coblentz and Baber (1987) did show that feral pigs have an adverse effect on several of the native animal species in their study area. It is apparent that changes in biodiversity can impact disease transmission and primarily through reduction of dead-end hosts, introduced alien species (vectors, reservoirs, hosts or pathogens), or increases in susceptible populations. The simple fact of the matter is that attempts to reduce transmission

nearly always involves the removal of, reduction of or negative impact on one or more vital components in the transmission cycle.

The research described herein will detail findings on the convergence of the invasive feral pig (*Sus scrofa domesticus*), native Texas tick fauna and the microbes they transmit as they occurred in the different Texas ecoregions.

CHAPTER II

SPATIAL AND TEMPORAL OCCURRENCE OF TICKS ASSOCIATED WITH FERAL SWINE IN TEXAS

Feral swine (*Sus scrofa domesticus),* European wild boar (*Sus scrofa scrofa*), and their crosses have dramatically increased in numbers across the United States in recent years, and as of 2007 the number of feral swine in Texas alone was conservatively estimated to have increased from 1M to 2M individuals (Burns 2007). The feral pig is listed by The World Conservation Union (IUCN) as one of the top 100 worst invasive alien species. The feral pig is one of only 14 mammals listed and one of two selected for specific discussion in the IUCN publication "100 of the World's Worst Invasive Alien Species" (Lowe et al. 2000).

There is little difference in the reproductive biology of the subspecies with regard to sexual maturation, litter size, and mating habits in the wild (Graves 1984). Males reach puberty between five and seven months of age, and puberty in females occurs at about 10 months of age on average (Sweeney et al. 1979, Mauget 1991). Litter size tends to be slightly higher in *Sus scrofa domesticus* and crosses between *S. domesticus* and *S. scrofa*. Litter sizes, for European wild boar, range from 4.5-6.3 (Mauget 1991) and 4.8-7.5, for feral swine (Sweeney et al. 1979). Litter size in younger sows tends to be smaller and increase following the first farrowing. Sow gestation period is 114 days making them capable of reproducing three times in a 14 month period, allowing for weaning. However, the majority of sows only reproduce once per year with multiple farrowing peaks occuring throughout the year (Sweeney et al. 1979, Taylor et al. 1998, Hanson and Karstad, 1959). Also, it has been noted in both *S. scorfa domesticus* and *S. scrofa scrofa* that resource availability and locality play a pivotal role in annual and seasonal breeding parameters (Bieber and Ruf 2005, Graves 1984). With this reproductive potential, in addition to

an average life expectancy of five years in the wild (Taylor 1991), there is the opportunity for large populations of this likely host to develop over a short period of time.

Anecdotal accounts from various sources, e.g. hunters, government trappers and landowners, indicate that heavy ectoparasite infestations on feral swine are common place across Texas. The substantial increase in numbers of this potential host of vector arthropods and reservoir potential of zoonotic agents is of key importance to those involved in investigating and predicting the public and animal health risks of diseases. Little is known about the role of feral swine as a host for arthropod vectors of zoonotic agents, the reservoir host potential of feral swine for zoonotic pathogens, or the transmission dynamics of such relationships in the United States.

Published data on arthropod infestations of feral swine in the United States, specifically Texas, are limited, and the published data are highly restrictive with regard to seasonality and spatial occurrence. Greiner et al. (1984) would be an exception. However, the study lacked methodological rigor in that pigs were inspected alive without aid of sedation. Other existing data are limited to the occasional field report supplied by government trappers, where there is likely to be substantial variation due to the sampling biases of the individual, or highly local and short duration studies, for example Coombs and Springer (1974).

Other literature available in the area of tick spices found on pigs are the Allan et al. (2001) feral pig study targeted naturally infected indigenous vectors of the causative agent of heartwater, *Ehrlichia ruminantium*. Greiner et al. (1984) were investigating African Swine Fever virus and the indigenous vectors from the genus *Ornithodoros*. Smith et al. (1982), on the other hand, conducted a broad survey of feral swine associated parasites, both internal and external, in the southeastern United States, but the study relied on small sample sizes from several locales from January 1979 through November 1980; the study was published just prior to

family of tick, *Ixodidae,* being shown as a competent vector of *Borrelia* species so no attention was given to the possible vector species in that family. Henry and Conley (1970) concentrated only on southern Appalachia and specifically on the introduced Eurasian boar population. Hanson and Karstad's 1959 paper reviewed what was known at the time with regard to the overall biology of feral swine in the southeastern US. Prior publications only mention incidental collections of arthropods from swine without clarification of the status of domestication e.g. (Cooley and Kohls 1944).

Ruiz-Fons et al. (2006) reported seven tick species from several provinces of Spain that were collected from Eurasian wild boar; *Dermacentor marginatus, D. reticulatus, Hyalomma excavatum, H. lusitanicum, H. m. marginatum, Rhipicephalus bursa,* and *R. sanguineus.* All of these are three-host tick species. Wild ungulates have become a major source of income for many agrarian provinces of Spain (Ruiz-Fons 2006). The possibility of adverse effects on this income that might result from an increased incidence of zoonotic diseases prompted a series of studies supported by the Instituto de Investigacio´n en Recursos Cinege´ticos including De la Fuente (2004), Ruiz-Fons (2006) and Ortuno et al. (2007). Even though the studies conducted in Spain reported data of tick species on boar in different geographies they have yet to undertake extensive and rigorous serological testing for previous exposure to many tick-borne zoonotic etiological agents. However, they are sufficient in warranting further investigation into the role of swine, feral pigs and wild boar, in tick-borne zoonotic pathogens.

The study described here was designed to test the hypothesis that feral swine may be an important component in the maintenance and dispersal of ticks in Texas. It was expected that diversity of tick species and the number individuals per species parasitizing feral swine would vary across the Texas landscape. This assumption was based on the dynamics of each tick

species' known host associations as well as habitat requirements of each species and that of feral swine.

Study objectives intended to test this hypothesis are: 1) to establish a record of tick species associated with feral pigs in Texas; 2) to compare the geographical and temporal distributions of individual tick species found in association with feral pigs; 3) determine whether tick assemblages on feral pigs vary in selected ecoregions of Texas.

Materials and Methods

Study Sites

There were two primary sites of investigation, Camp Bullis, Texas and Welder Wildlife Refuge, Sinton, Texas. Camp Bullis lies predominantly within the Edwards Plateau ecoregion but is in close proximity to both the Blackland Prairie and South Texas Plains ecoregions, and Welder Wildlife Refuge (WWR) lies along the transition area of the Gulf Prairies and Marshes and South Texas Plains ecoregions. Additional samples were taken from other ecoregions in association with Texas Agrilife Extension Service - Wildlife Services and other collaborators whenever possible.

Camp Bullis Military Reserve: Camp Bullis Military Reservation (CBMR) is a 12,300-ha military training facility that sits on the eastern edge of the Edwards Plateau ecoregion known as the Balcones Canyon Lands. The Edwards Plateau ecoregion land features and plant life have been previously described in detail (Correll and Johnston 1970). Briefly, the land features rolling plains and deep valleys with very shallow soils except for along drainages. There are many karst features throughout the CBMR as it sits atop a portion of the Edwards aquifer recharge zone. The average rainfall is approximately 83.8cm per year with seasonal peaks in May to June and September. Daily average temperatures are between 15°C December to

February and 35°C June to August. Geolocation data for feral pig movements and land use are nonexistent for this area.

Welder Wildlife Refuge: The WWR lies in a transitional zone between the South Texas Plains and the Gulf Prairies and Marshes vegetational communities (Drawe et al. 1978). Because of this, the site description will be more detailed. This study was conducted on the 3,160 ha Welder Wildlife Refuge in San Patricio County, 50 km north of Corpus Christi, Texas. The climate is humid, subtropical with hot summers (*x* July temperature = 30°C) and cool winters (*x* January temperature = 14°C). The 53-year average annual rainfall was 92.2 cm. Monthly rainfall means show a bi-modal distribution with peaks in May-June and September. Previous data collected on WWR show feral pigs on the refuge to have an estimated average home range of 126ha ± 26ha (Campbell et al. 2010). Deck et al. in 2006, using geolocation and video data, showed similar home range data as Campbell et al. in 2010, and that feral pigs in the South Texas Plains ecoregion land type use places them in riparian zones 70% of the time.

There are 5 major habitat types (mesquite, chaparral, grassland, live oak, riparian) that comprise 90% of the WWR. The mesquite habitat type was on flat poorly drained Victoria Clay soils. Canopy cover ranged from 16-34 percent in 1987 (Drawe et al. 1991). The vegetation was comprised of honey mesquite (*Prosopis glandulosa*) interspersed with granjeno (*Celtis pallida*), Texas persimmon (*Diospyros texana*), lime pricklyash (*Zanthoxylum fagara*) and larger expanses of mixed grasses. Huisache (*Acacia smallii*) has become a major invader. Dominant grasses were Texas wintergrass (*Stipa leucotricha*), knotroot bristlegrass (*Setaria geniculata*), silver bluestem (*Schizachyrium scoparium* var. *frequens*), and vine mesquite (*Panicum obtusum*). Dominant forbs included western ragweed (*Ambrosia cumanensis*), ruellia sp., and prairie coneflower (*Ratibida columnifera*).

The mesquite grades into the chaparral habitat type which has better drained clay soils and increased density and canopy cover of brush. Canopy cover ranged from 39 to 82 percent in 1987 (Drawe et al. 1991). The main species comprising the chaparral were lime pricklyash, brazil (*Condalia hookeri*), Texas persimmon, agarito (*Mahonia trifoliolata*), and granjeno. Herbaceous vegetation was similar to the mesquite habitat type.

The grassland communities were found on sandy soils. Few brush or tree species were present and the major herbacious species were seacoast bluestem (*Schizachyrium scoparium* var. *littorale*), *Dicanthelium oligosanthes*, thin paspalum (*Paspalum setaceum*), *Croton* spp, silverleaf sunflower (*Helianthus argophyllus*), and skunk daisy (*Verbesina encelioides*). Canopy cover ranged from 0 to 8 percent in 1987 (Drawe et al. 1991).

The live oak habitat is a three-layered community with live oak (*Quercus virginiana*), chaparral, and bunchgrass found on sandy loam soils. With over lapping layers of brush and live oak, the percent canopy cover ranged from 97 to 113 from 1982-87 (Drawe et al. 1991). The chaparral component is similar to the above described chaparral habitat type. The herbacious vegetation is similar to that on sandy soils with additional shade tolerant species such as turk's cap (*Lilium michauxii*) and mistflower (*Eupatorium coelestinum*). Seacoast bluestem, brownseed paspalum (*Paspalum plicatulum*), and windmillgrasses (*Cloris* spp.) were major grass species present.

The riparian habitat type was found along the Aransas River and was dominated by larger trees including hackberry, elm, pecan (*Carya illinioensis*), and anacua (*Ehretia anacua*). Mustang grape (*Vitis mustangensis*) vines drape the trees. A shrub understory was made up of lime pricklyash, Texas persimmon, granjeno, and agrarito. Southwestern bristlegrass (*Setaria scheelei*) and shade tolerant plants such as turk's cap and Virginia wildrye (*Elymus virginicus*)

are found under the canopy of the larger trees. Man-made tanks or natural lakes and wetlands along the river provide water in all seasons, except during drouths.

Feral Pig Collections

Members of the collaboration harvested feral pigs from CBMR and WWR throughout the year in an attempt to meet a 25-pig target for each site per seasonal quarter or for a total of 100 pigs per site annually (Texas A&M University IACUC Animal Use Protocol (AUP) #2008-131). This sample size generated an estimation of tick loads on local pig populations for both gender and age classes (juvenile and adult) throughout the year. With no background data to base sample sizes on, the target sample size used in this study served merely as an *a priori* starting point.

Pigs were trapped using corral and box traps. Trap designs were based on traps used by Texas Agrilife Extension Service - Wildlife Services. Traps were pre-baited prior to actual trap dates to allow pigs to become habituated to the availability of the bait and to the traps being in their surroundings. Pre-baiting, providing bait with trap doors tied in the open position, is a known and common tactic used to maximize capture success (Schulyer et al. 2002). The day prior to trapping, traps were set after midday and checked as soon as possible the following day based on lighting and weather conditions. On overcast days, traps were checked later to avoid disturbing any pigs that might still be feeding.

Pigs were euthanized using a .22 caliber handgun or rifle with low velocity ammunition and processed for tick collection in accordance with AUP #2008-131. The .22 caliber low velocity ammunition limited bleeding into the ears where numerous tick species are known to infest hosts. Samples collected in conjunction with Texas Agrilife Extension Service - Wildlife Services were taken from pigs harvested by aerial shooting. Immediately upon euthanasia

individual pigs were given a unique accession number that was assigned to all vials of arthropods collected from the animal.

Arthropod Collection and Identification

Immature ticks collected from each pig were placed in plastic baggies with moistened paper toweling, deposited into a cooler for storage until transported back to the Tick Research Laboratory (TRL), Texas A&M University where they were allowed to molt for more accurate species level identification. Adult tick specimens were deposited into 80% EtOH, transported to the same lab and indentified to species (Keirans and Clifford 1978, Cooley 1946, Yunker et al. 1986, Keirans and Litwak 1989, Estrada-Pena et al. 2005, Cooley and Kohls 1944, Robinson 1926, Keirans and Durden 1998, Pratt and Stojanovich 1969). Fleas and lice are retained at the tick laboratory for future studies. The unused ticks were placed in individual vials, by species and accession number, with 80% EtOH and held for future analysis as needed.

Results

The number of pigs harvested between June 2008 and June 2010 from all sources was 432. The total pig harvest by TRL personnel breakdown as follows: (152) WWR, San Patricio County, Texas; (17) Camp Bullis Military Installation, Bexar County, Texas; (26) Brazos County, Texas; and 20 from the border area of Liberty and Harris Counties. Additional samples from Texas Agrilife Extension Service - Wildlife Services totaled 217 animals. Harvests were from 10 ecoregions and 29 Texas counties (Figures 1A and 1B). The percentage of animals infested with ticks for all seasons was 75% for pigs harvested by TRL personnel and 62% for pigs harvested by Texas Agrilife Extension Service - Wildlife Services. Pigs photographed on Camp Bullis, using infrared game cameras, indicated they roamed most of the 11,000ha military reserve with no easily discernable pattern. Trapping efforts, days trapped, for Camp Bullis were as much as three times that of the days trapped at WWR.

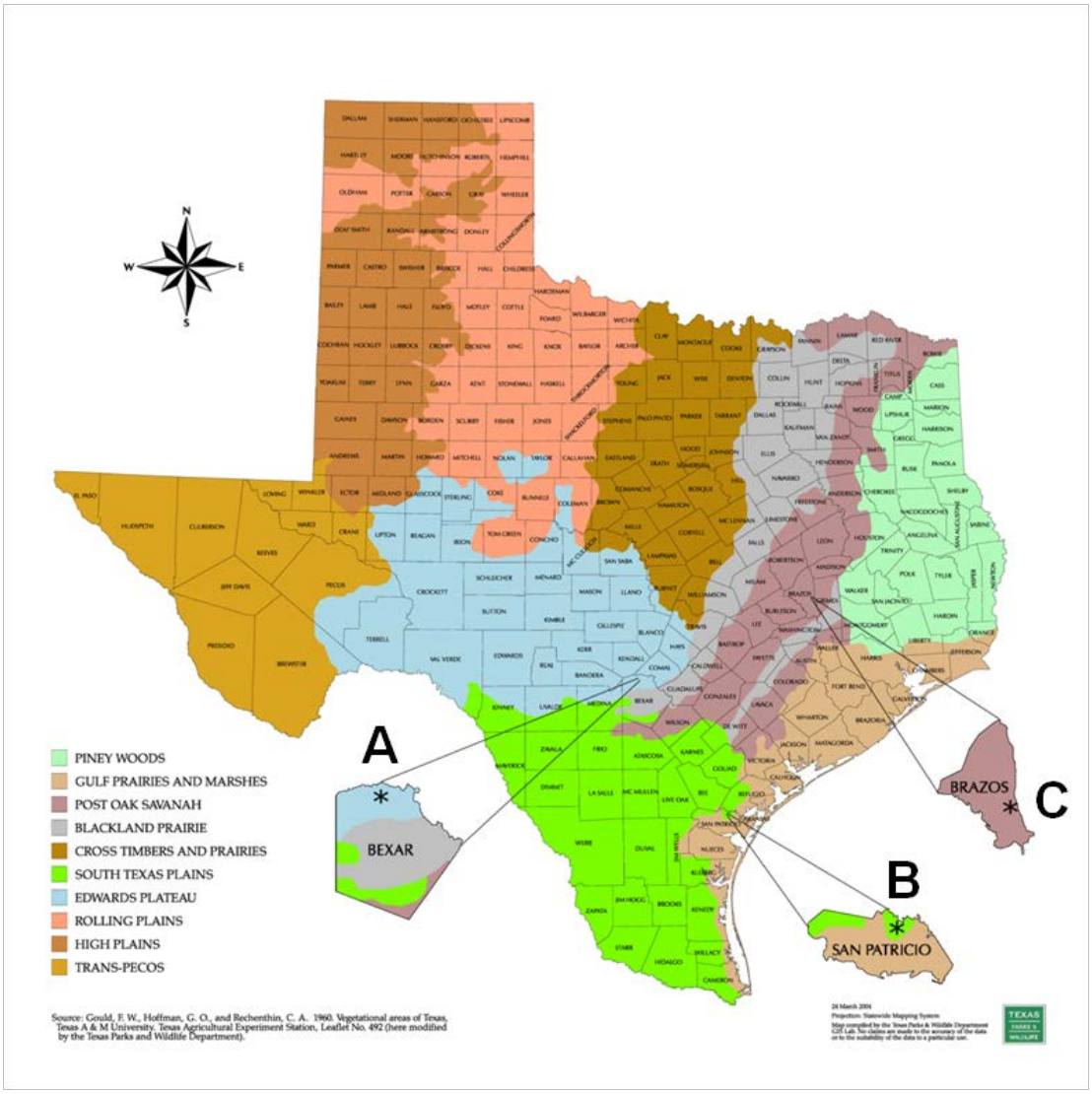


Figure 1A Texas map depicting the ecoregions of Texas with pull outs for sites used as part of this study.

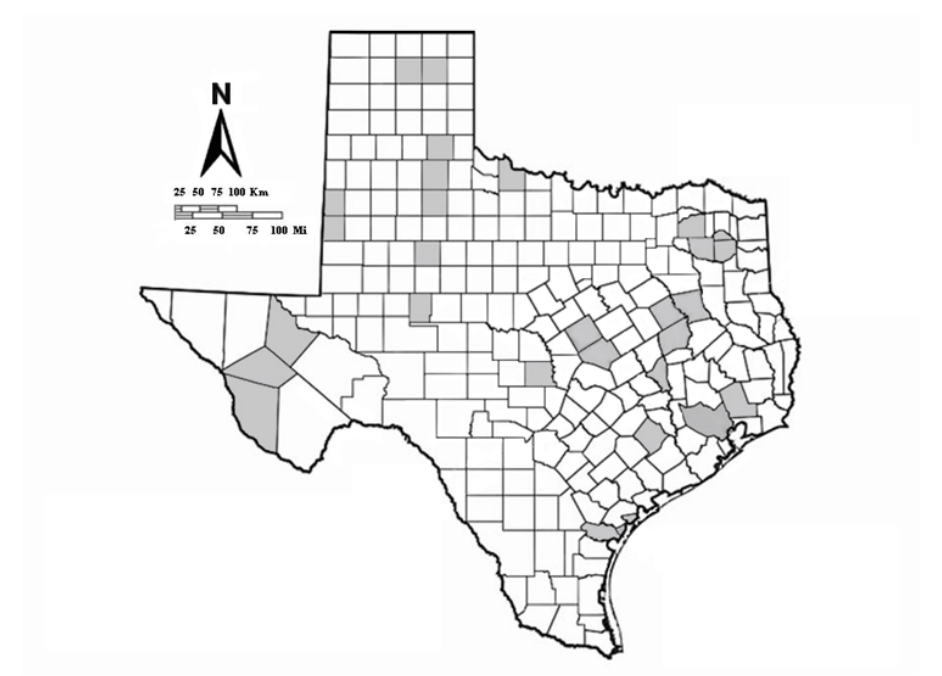


Figure 1B Texas county map of Texas counties where pigs were harvested between June 2007 and June 2010 shaded in gray.

Seven species of Ixodid ticks, from three genera, were collected over the course of three years of study; *A. americanum, A. cajennense,* and *A. maculatum*; *D. albipictus, D. halli,* and *D. variabilis*; and *I. scapularis* (Table 1). Three of the species, *A. cajennense, A. maculatum,* and *I. scapularis*, reported here have been reported previously from feral swine in Texas (denoted in Table 1). Twenty-three *A. cajennense* nymphs were collected from animals on the WWR and allowed to molt to the adult stage at the TRL facility for identification to the species. *Amblyomma americanum* nymphs were collected from Anderson, Bell, Brazos, Coryell, Hopkins, and Wilbarger counties. Other arthropods, *Pulex* fleas and *Haematopinus* lice, were also taken from feral pigs. Flea counts, at times, where indeterminable.

Table 1. Tick species recovered from feral swine harvested in the state of Texas between June of 2008 and June of 2010 listed by genus.

Amblyomma	Dermacentor	Ixodes
*A. cajennense**+	*D. variabilis*	*I. scapularis**
*A. maculatum**+	*D. halli*	
A. americanum+	*D. albipictus*	

* Denotes tick species recovered in previous studies.
\+ Denotes tick species collected as nymphs in this study.

It was hypothesized that tick diversity and tick burdens would vary between the two primary sites based on the influences of their primary and adjacent ecoregions. Camp Bullis Military Reservation produced five species of ticks, four with 3-host and one with 1-host biology, and had a lower percentage of infested animals than WWR where only four species of ticks were collected all of which utilize 3-host biology (Table 2). Depending on seasonal activity, the tick burden at WWR could be estimated at 1000 ticks per animal or more on many individuals during the month May-September with the predominant species being *A. cajennense*. At CBMR, the highest number of ticks taken from an individual pig was nine. The average number of ticks collected per animal on WWR varied based on seasonal activity of the species but also on the social habits of the individual pig. For example, the tick loads for the predominant species collected at WWR, *A. cajennense*, for all animals with ticks ranged from 53.7 ticks per animal during higher tick activity periods and down to 2.2 ticks per animal during lower activity periods. The most commonly collected tick species at CMBR was *A. americanum*, which averaged one tick collected per infested animal. Figure 2 shows the average number of ticks collected from an age by gender class, as well as for the social activity variation of boar pigs harvested from WWR. The population makeup was 32% boar, 26% sow, 15% shoat, and 23% gilt. The switch in dominant gender is no doubt from breeding pressure placed on the females as the age to adult sow.

Table 2	Summary of total pigs harvested between June 2008 and June 2010, percentage of pigs infested, tick assemblages, number collected for each species and months of greatest numbers of ticks collected for each Texas ecoregion.				
Ecoregion	Pigs harvested	Percent Infested	Tick Species	Ticks Collect	Months Collected
Piney Woods	43	66%	*A. americanum*	19	July
			A. maculatum	141	
			D. variabilis	8	
Gulf Prairies and Marshes	167	75%	*A. cajennense*	2312	May – Sep
			A. maculatum	371	May – Sep
			D. variabilis	170	Jan
			D. halli	1	Jan
Post Oak Savannah	43	63%	*A. americanum*	198	Jan – Dec
			A. maculatum	208	Mar – Jul
			D. variabilis	14	Jun
			I. scapularis	5	Mar
Blackland Prairie	76	44%	*A. americanum*	19	Jul – Aug
			A. maculatum	23	
			D. variabilis	11	
Cross Timbers and Prairies	189	22%	*A. americanum*	6	Apr – May
			A. maculatum	79	Jun – Aug
			D. variabilis	24	Mar – June
			I. scapularis	8	Jan – May
South Texas Plains	Included in Gulf Prairies and Marshes				
Edwards Plateau	23	65%	*D. variabilis*	4	Aug – Sep
			A. maculatum	10	Jun – Sep
			A. americanum	6	Apr – Jun
			D. albipictus	1	Mar
			I. scapularis	1	Mar
Rolling Plains	73	82%	*A. maculatum*	4	May, Sep – Oct
			D. variabilis	364	Mar – Sep
High Plains	14	7%	*D. variabilis*	5	May
Trans-Pecos	7	0	N/A		N/A

Gulf Prairies & Marshes and South Texas Plains ecoregions were combined since WWR sits on the transition zone between the two ecoregions and feral swine utilize both areas.
High Plains and Trans-Pecos ecoregions had very low pig harvests and were outside of peak activity for many tick species.
Cross Timbers and Prairies samples were split among other research groups invalidating the percentage of pigs infested estimate.
Ninety-three of the *D. variabilis* included in the Rolling Plains count came from counties that lie in the transition zone of the Rolling Plains and High Plains ecoregions.

The average number of ticks from solitary infested boars averaged approximately 12.2 ticks per animal as compared to gregarious boars at 33.8 ticks per animal with ticks. Collections were biased with regard to the infestation level of the individual pig. An attempt to collect all available ticks was the norm for pigs with lower infestation levels, less than 100 ticks. Whereas it was not practical to attempt to collect all ticks from animals with heavy infestation levels, e.g. several hundred.

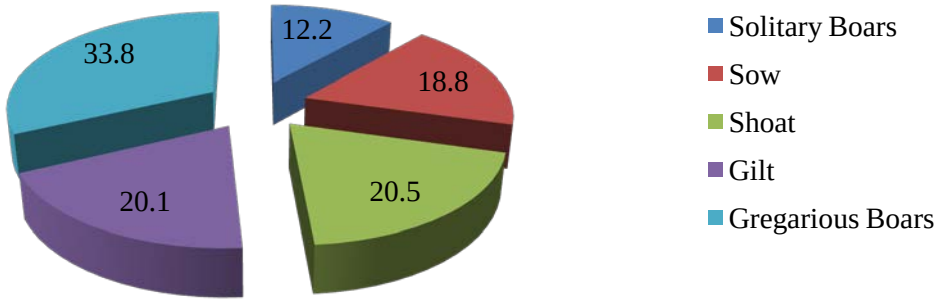


Figure 2 Welder Wildlilfe Refuge average number of ticks collected from hosts found to have ticks. These data are based on age classes as well as social status for boar pigs. Pigs without ticks were not included to mitigate the effect of tick seaso

Amblyomma cajennense, *A. maculatum*, and *D. variabilis* made up the predominant species of ticks collected from feral swine on the WWR, Gulf Prairies and Marshes and South Texas Plains ecoregions. The representative species of Camp Bullis, Edwards Plateau ecoregion, were *A. americanum, A. maculatum, D. variabilis, I. scapularis* and *D. albipictus*. However, in Camp, Hopkins and Wood counties, the predominant species collected were *A. maculatum, A. americanum* and *D. variabilis*, and the species collection records show *A.*

americanum and *A. maculatum* collections decreasing in numbers the further west in Texas the hosts were harvested with *D. variabilis* being collected from the Texas and New Mexico border. This shows assemblages do vary with ecoregion.

Discussion

The objectives of this study were: 1) to establish a record of tick species associated with feral pigs in Texas; 2) to compare the geographical and temporal distributions of individual tick species found in association with feral pigs to the currently accepted distributions in Texas; 3) to determine whether the tick assemblages on feral pigs vary in different ecoregions of Texas. Based on our collection records these objectives appear to have been met. However, it must be pointed out that these data are not all inclusive. For example, it is extremely difficult to ascertain parasitism by adult ticks of the family Argasidae on any host by collection records from the host.

Several important vector species were shown to feed on feral swine with relative frequency and at least three species will feed on feral swine in the nymphal stage. While there does not seem to be any effect on the seasonality of the tick species on feral swine, the collection of *D. variabilis* and *A. maculatum* outside their accepted ranges does lend credence to the hypothesis that feral pigs may be moving and establishing important vector species in new areas. Many studies have shown that feral pigs do impact the ecology of areas where they become established. This type of behavior may be creating microhabitat changes that allow vector and nuisance tick species to expand their respective ranges with the aid of their host. At the very minimum and unarguably, the pigs are serving as additional hosts for these species further complicating any control efforts targeting vector or nuisance tick species.

Figure A1 depicts the geographic area over which *A. maculatum* and *D. variabilis* were collected in association with feral swine. These collections support similar collection data for *A. maculatum* of the Texas Animal Health Commission's livestock surveys. Figure A2 shows the

most recently published distributions of these two species (Teel and Hurley 2010). These are based primarily on Texas Animal Health Commission records taken from domestic livestock. These areas are noticeably different from the distribution maps posted by the Centers for Disease Control on their website and what is generally accepted as the respective distributions of the two species. The additional species collected from feral swine showed no difference in the expected geographic zones (A3-A5).

Dermacentor halli and *D. albipictus* were collected during the course of this study. *Dermacentor halli* is a little known tick and is generally thought to be associated with javelina (*Pecari tajacu*) and was only collected from the WWR on one occasion, but the specimen was a partially engorged female. *Dermacentor albipictus* is a one host species and, usually without exception, spends all life stages on a single host and was only collected from Camp Bullis military installation in a single instance. This specimen was also a partially fed female. In all likelihood, had the females not been detached they would have fed successfully and been able to produce offspring assuming successful mating had occurred.

Feral pigs continue to become established across the United States and will likely serve as an additional host for an increasing number of tick species and will likely serve as a means of relocation and establishment in new geographical regions for some of these species which are currently limited by other environmental constraints. Already in the United States we are seeing peridomestic populations of feral pigs rise to levels requiring control efforts. These particular feral pigs are just as hard to trap as sylvatic, but control efforts are further complicated by groups and individuals concerned about the humane treatment of the invasive species. The potential for newly emerging or reemerging zoonotic diseases is developing all across the United States.

CHAPTER III

SEROPREVALENCE IN TEXAS FERAL SWINE TO *RICKETTSIA, EHRLICHIA,* AND *BORRELIA*

Feral swine (*Sus scrofa domesticus),* European wild boar (*Sus scrofa scrofa*), and their crosses, because of their reproductive capacity, have had large population increases across North America since their introduction in the 1500s and now cover most of the United States. The population in Texas was estimated to be at or greater than 2M individuals (Burns 2007). Feral pigs are known to alter natural ecologies and to cause costly damage to both urban and rural managed landscapes in parts of the world where they become established. A series of studies was initiated based on the hypothesis that feral swine are likely to serve as hosts for ticks on a landscape where the pigs are newly introduced. Chapter I reports feral swine in Texas have been shown to serve as hosts for seven species of ticks, and the percentage of animals infested may be as high as 75%. The prominent vector species were *Ixodes scapularis*, *Dermacentor variabilis*, *Amblyomma americanum, A. cajennense* and *A. maculatum*. The outcome of that study further developed the hypothesis that feral swine are involved with the tick-borne pathogen transmission cycles endemic to Texas.

The species of ticks listed above indicated that the three genera of tick-borne bacteria that might best assess the exposure of Texas feral swine to tick-borne pathogens were *Rickettsia, Ehrlichia*, and *Borrelia*. The decision to conduct a serosurvey at the genus level was based on the tick species known to infest feral swine and the pathogens they serve as vectors for but more importantly based on the unknown factors. Lin et al. (2005) reported a novel *Borrelia* in *D. variabilis* from Webb County, Texas, and *A. cajennense* is considered a primary vector of *Rickettsia rickettsii* in the southern hemisphere but is hardly mentioned in North American cases

of Rocky Mountain spotted fever. These genera of tick-borne bacteria were considered to be relevant since at least one species that causes human disease from each of the three genera as well as its vector tick species are known in Texas, and now the vector has a collection record from Texas feral swine.

Borrelia spirochetes are corkscrew shaped flagellated highly motile eubacteria of the family Spirochaetaceae. The *Borrelia* species of medical importance are generally divided into one of two types of Borreliosis, Lyme Borreliosis or Relapsing Fever Borreliosis. The causative agent of Lyme Borreliosis was originally confined to the single species of *Borrelia burgdorferi*. However, the more pathogenic forms have, over time, been subdivided into three species, *B. burgdorferi*, *Borrelia garinii*, and *Borrelia afzelii* (Steere et al. 2005). Texas confirmed 363 cases of Lyme Borreliosis, based on case definitions established by the Centers for Disease Control, between 1986 and 1992 (Rawlings and Teltow 1994). The primary vector species, *Ixodes scapularis*, is a common Texas three-host tick species. Tick-borne relapsing fever etiological agents, e.g. *Borrelia turicatae*, are also known in Texas since the 1920s (Dworkin et al. 2008) as is the vector tick species, *Ornithodoros turicata* (Cooley and Kohls 1944). The relapsing fever form of Borreliosis is further divided into either tick-borne relapsing fever (TBRF) or louse-borne relapsing fever (LBRF). There is only one species of Borrelia currently accepted as associated with LBRF, *Borrelia recurrentis* (Barbour 2005). On the other hand, there are several species of TBRF causative agents. *Borrelia turicatae* is a TBRF agent most likely to be relevant to a serological study of feral pigs in Texas due to the pathogen occurrence and the feeding habits of the vector, *O. turicata*. The vector, *O. turicata*, is an extremely fast feeder among ticks and is not likely to be collected from the host making it very difficult to elucidate the particulars of any transmission that might be occurring through host records. By assessing previous exposure to *B. turicatae* it might at least be possible to determine if any such

transmission possibly exists. Finally, there are at least three other pathogenic Borrelia transmission cycles in Texas that might possibly be affected by the presence of feral pigs on the Texas landscape. These are *Borrelia lonestari, Borrelia hermsii* and a novel *Borrelia* recently recovered from *D. variabilis* in Webb County, Texas (Lin 2005).

Similarly the causative agent of human monocytic ehrlichiosis (HME), *Ehrlichia chaffeensis,* is known in Texas. McQuiston et al. (1999) reported an annual incidence rate of 0.20 clinically defined cases per million population. The accepted vector of *E. chaffeensis, Amblyomma americanum,* is common to Texas as well (Cooley and Kohls 1944). *Ehrlichia chaffeensis* is an intracellular obligate, immotile, gram-negative bacteria species that displays tropism towards white blood cells in infected humans (Dawson et al. 2005). Until recently *E. chaffeensis* belonged to the order Rickettsiales. *Ehrlichia chaffeensis,* along with *Cowdria ruminantium, E. canis, E. ewingii* and *E. muris,* now belongs to the genus *Ehrlichia* (Dawson et al. 2005). The adult stage of the primary vector tick species, *A. americanum,* is generally considered to have broad host range and is the primary vector species of *E. chaffeensis*. Rawlings and Teltow (1994) reported *A. americanum* to be the most common tick collected from Texas state parks. It is reasonable then to believe that across most of Texas feral swine have been exposed to *A. americanum* and with some diminishing probability to *E. chaffeensis*.

The annual incidence rate in humans from Texas for clinically defined Rocky Mountain Spotted Fever (RMSF) is very similar to that of HME, 0.30 cases per million population (Treadwell et al. 2000). A vector tick species for the causative bacterial agent of RMSF, *Rickettsia rickettsii,* is *Dermacentor variabilis* another three-host tick species common to Texas (Yunker et al. 1986). Rickettsial organisms, like *Ehrlichia* species, are rod shaped intracellular obligate immotile bacteria belonging to the class α-Proteobacteria. However, *Rickettsia* exhibit endothelial cell tropism versus the seeming attraction of *Ehrlichia* to reticuloendothelial cells

(Macaluso and Azad 2005). The vector of *R. rickettsii* in Texas, *D. variabilis,* is another common three-host tick with a broad host range (Bishopp and Trembley 1945). In fact, Bishopp and Trembley (1945) reported records of *D. variabilis* on "hog".

Materials and Methods

Study Sites

There were two primary sites of investigation, Camp Bullis, Texas and Welder Wildlife Refuge, Sinton, Texas. Camp Bullis lies predominantly within the Edwards Plateau ecoregion but is in close proximity to both the Blackland Prairie and South Texas Plains ecoregions, and Welder Wildlife Refuge lies along the transition area of the Gulf Prairies and Marshes and South Texas Plains ecoregions. Sites were described in detail previously. Additional samples were taken from other regions in association with Texas Agrilife Extension Service - Wildlife Services and other collaborators whenever possible.

Feral Pig Collections

Trained personnel harvested feral pigs in accordance with Texas A&M IACUC Animal Use Protocol #2008-131 guidelines using methods previously described (Chapter I).

Whole blood and serum were collected immediately after euthanasia to avoid coagulation or degradation of the samples. Blood samples were taken by intrathoracic cardiac puncture per the approved animal use protocol. Three Vacutainer® blood collection tubes were used, Bectin / Dickinson tubes and catalog numbers EDTA serum separator (367812), ACD solution B (364816)**,** and molecular grade serum separator (367986). The molecular grade serum separator, item number 367986, was discontinued due to incompatibility with rigors of field conditions. Subsequently, two traditional EDTA, 367812, tubes were used instead.

Previous Exposure Assessment by Enzyme-Linked Immunosorbent Assay (ELISA)

Bacteria Stocks: *Rickettsia rickettsii* was supplied by Dr. Don Bouyer of the University of Texas Medical Branch (UTMB), Galveston, TX. Bacteria were allowed to replicate in HeLa cells until the infection rate reached 80%. Bacteria were then heat killed at 90°c for 95 minutes. *Ehrlichia chaffeensis* was also supplied by Dr. Don Bouyer of UTMB. *Ehrlichia chaffeensis* was replicated and harvested in the same manner as the *R. rickettsii*. However, *E. chaffeensis* was heat killed at 50°C for 30 minutes.

Borrelia turicatae was supplied by Dr. Tom Schwan, Chief of Laboratory of Zoonotic Pathogens of the NIAID-NIH, Rocky Mountain Laboratories, Hamilton, MT. *Borrelia* spirochetes were produced using HeLa cells and harvested at 75 – 80% infection rate. Bacteria were sonicated for cell disruption as described by Schwan et al. (1989).

Final protein concentrations for all pathogen lysates were ~ 1.5-1.6 mg/ml by Bradford protein assay using Labsystems Multiskan® Plus plate reader.

Antibody Production: Three groups of three weanling domestic stock pigs, between 25 and 30lbs, were used to develop antibodies against *R. rickettsii*, *E. chaffeensis*, and *B. burgdorferi* under Amendment A to Texas A&M University AUP#2007-85. Weanlings were farrowed and reared to weaning age and proper weight under environmentally controlled conditions without exposure to ticks or tick-borne pathogens. At the time of initial exposure, pigs were anesthetized using a solution of Xylazine / Telazol at a dosage of 100mg/ml of Xylazine and 25mg/ml of Telazol. The IM delivered dosage is 8mg/kg for Xylazine and 2mg/ml for Telazol. Two 20ml vials of serum were taken from each live pig prior to exposure to serve as negative controls in enzyme-linked immunosorbent assays (ELISA). Each pig was then given one initial intramuscular dose of 0.25ml of the respective crude bacterial lysate in each hip. Thirteen days later pigs were re-anesthetized based on the same protocol. They were then given

booster injections with 0.25ml of the respective lysate in the commercial adjuvant TiterMax® Gold (CytRx, Los Angeles, CA). The success of the antibody production was determined based on the absorbance readings of the ELISA assays controls, negative and positive, discussed below.

ELISA Assay: Feral swine sera were tested for IgG response to *Rickettsia* and *Ehrlichia* using whole cell lysates of *R. rickettsii* and *E. chaffeensis*. Sera were also assayed for *Borrelia* exposure using purified recombinant glycerophsodiester phosphodiesterase (rGlpQ) cloned from *Borrelia hermsii* as per established protocols (Schwan et al. 1996). This antigenic protein is conserved among the relapsing fever causing *Borrelia*, and is commonly used for the discrimination of this group of *Borrelia*. The 96-well plates used for the *Borrelia* assays were NI-NTA HisSorb nickel lined plates (Qiagen, Valencia, CA) and each well was coated with 100 µL of rGlpQ at a concentration of 10 µg / ml. The whole cell lysate assays were run on 96-well flat-bottom Immulon-2 plates (Dynatec Laboratories Inc, Alexandria, VA). Plates were coated with 50µL/well of either *Rickettsia* or *Ehrlichia* antigen and placed in moist chambers at 37°C overnight followed with five washing with PBS-Tween 20 (1X PBS, 0.05% Tween 20) prior to blocking. Final protein concentrations for phosphate buffered saline (PBS) suspended lysates was100 µg of antigen per milliliter. Blocking was accomplished using ELISA diluent (PBS, 0.5% horse serum, 0.05% Tween 20, 0.001% dextran sulfate) at 100 µL per well and incubated for 1 hour at 37°C on a platform shaker. Plates were washed five times and swine serum, at 1:200 dilution, was added to wells at 100 µL per well and incubated for 1hour at 37°C on a platform shaker. Plates were washed five times, and 100 µL of secondary antibody, horseradish peroxidase conjugated goat anti-pig (Thermo Scientific, Waltham, MA), was then added to each well at 1:5000 dilution, incubated at 37°C for 1hour and washed five times. Substrate, 50% 2, 2'-azino-di-(3-ethyl-benzthiazoline sulfonate), was added and incubated for ~ five minutes.

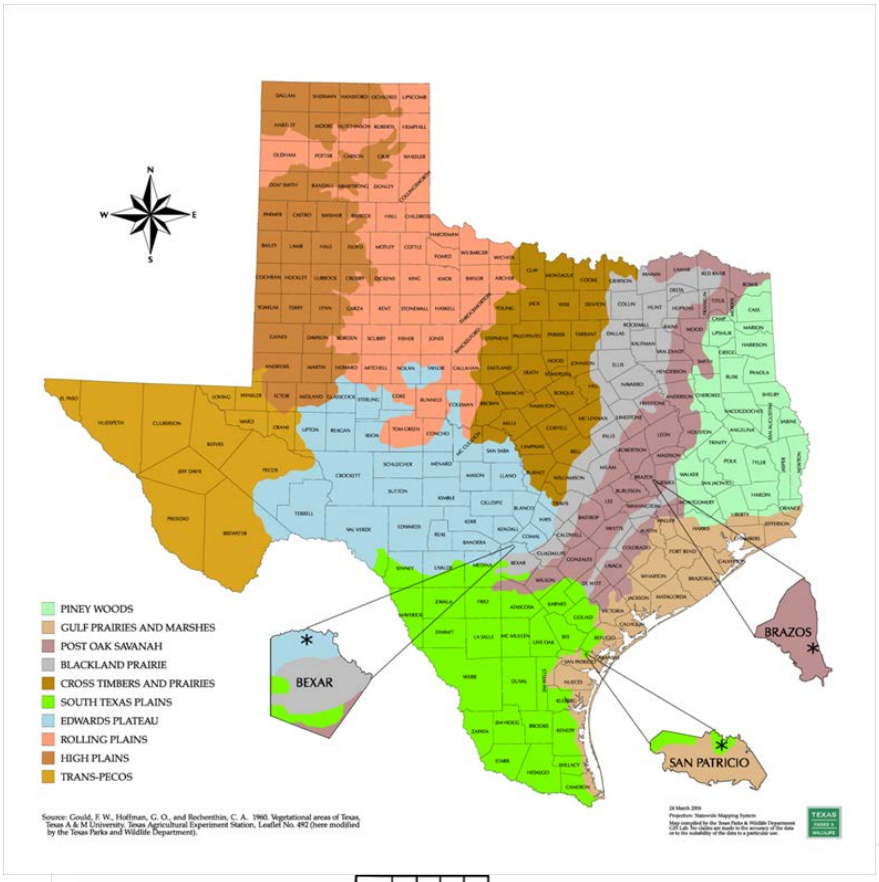
Times varied based on the rate at which each plate reacted. Plates were read at 405 nm with a Labsystems Multiskan® Plus plate reader (Thermo Scientific, Waltham, MA).

The bottom row of each plate was reserved for eight negative controls, three positive controls and a blank. Samples were considered positive if the respective absorbance reading was greater than the mean ($\bar{x}$) plus three times the standard deviation (SD), ($\bar{x}$ + 3 * SD), of the negative controls for that plate.

Western Blot: Select samples that showed antibody response to the rGlpQ of *B. hermsii* by ELISA were further processed and analyzed by sodium dodecyl sulfate (SDS) page gel and Western (immunoblot) blot. Twenty trios of Precision Plus Protein Kaleidoscope Standard ladder (Invitrogen, Carlsbad, CA), *B. turicatae* whole cell lysate and rGlpQ from *B. hermsii* were electrophoresed for 1.5hrs through 12% Tris-Glycine 1mm 10 well gel (Invitrogen) and transferred nitrocellulose membranes using iBlot dry blotting (Invitrogen). Membranes were blocked for 1hr per iBlot protocol, and were then removed, sealed in cellophane with the sera from samples referenced above at a 1:200 dilution for 1hr. Membranes were then probed with secondary antibody (goat anti-pig at a 1:5000 dilution), developed and analyzed per iBlot protocol. Samples were considered positive for exposure to *B. turicatae* if the sample was positive for rGlpQ in the third lane at approximately 50kd and showed multiple bands in the second lane with *B. turicatae* whole cell lysate.

Results

Nine-hundred fifty-six pigs were harvested and sera collected. Texas Agrilife Wildlife Services harvested 744 animals and Texas A&M University Tick Research Laboratory personnel harvested 212 feral swine. Feral pigs were taken from 63 Texas counties and all ten ecoregions (Figure 3). The population makeup for WWR was 32% boar, 26% sow, 15% shoat, and 23% gilt.



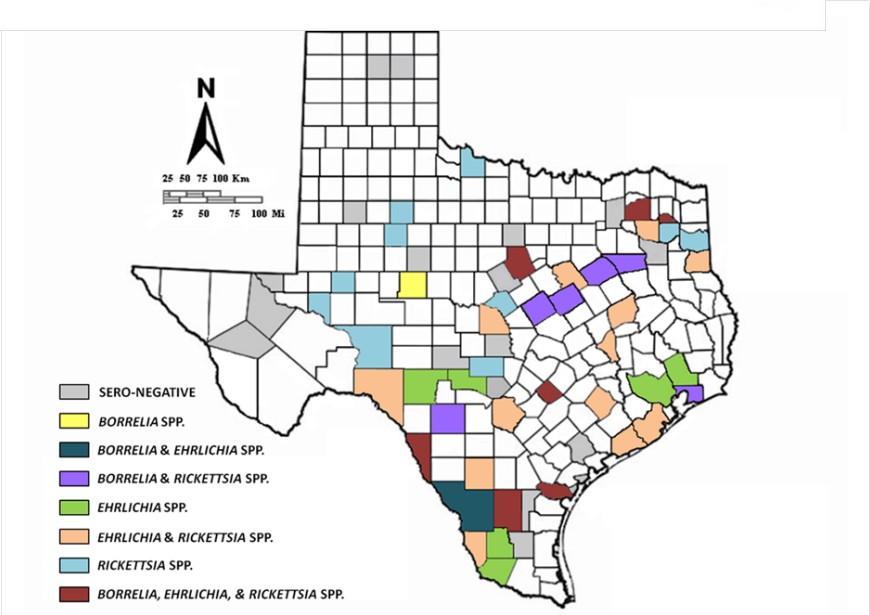


Figure 3 Texas County map illustrating the counties from which feral pig serum was analyzed and the possible assay outcomes from negative to positive for all three pathogens of interest.

Because of time and logistical constraints, samples analyzed for *Rickettsia* and *Ehrlichia* exposure by ELISA immunoassay were limited to 888 and 849 samples for previous exposure to relapsing fever causing *Borrelia*.

Antibody Production: Three pigs were inoculated with *R. rickettsii, E. chaffeensis,* or *B. turicatae* for three cohorts of three individuals each. The mean absorbance, at 405 nm, for positive sera was 0.591+/- 0.299, 0.365 +/- 0.236, and 0.197 +/- 0.112 for *Borrelia*, *Rickettsia* and *Ehrlichia*, respectively. Negative sera had mean absorbance readings of 0.256 +/-0.126 (*Borrelia*), 0.175 +/- 0.126 (*Rickettsia*), and 0.196 +/- 0.103 (*Ehrlichia*). Obviously, the *Borrelia* and *Rickettsia* cohorts showed a twofold increase in antibody response contrary to the virtual lack of response on the part of the *Ehrlichia* cohort. This was not necessarily a problem for the assay outcomes as the absorbance readings were compared to the means and standard deviations of the respective negative controls as described below.

ELISA: Samples deemed positive had absorbance readings greater than the mean ($\bar{x}$) plus three times the standard deviation (SD), ($\bar{x}$ + 3 * SD) for the plate on which the sample was analyzed. Feral pigs deemed positive for previous exposure to *Ehrlichia* or *Rickettsia* organisms were, 117 and 245, respectively. Eighteen pigs were shown to be positive for exposure to what is most likely a relapsing fever group *Borrelia* species an additional four being just below their respective cutoff absorbance readings. Forty-two pigs were positive for multiple organisms with 30 of these being positive for *Ehrlichia* and *Rickettsia*, 10 for *Rickettsia* and *Borrelia*, one for *Ehrlichia* and *Borrelia* and one for all three.

Geographic distribution across Texas was widespread for all three bacterial pathogens, though not necessarily contiguous. Figure 4A and 4B and Table 3 provide prevalence estimates and illustrate distribution of each pathogen by ecoregion. Eight of the 10 Texas

ecoregions produced feral swine positive for previous exposure to a *Rickettsia, Ehrlichia*, or *Borrelia* like organism (A6).

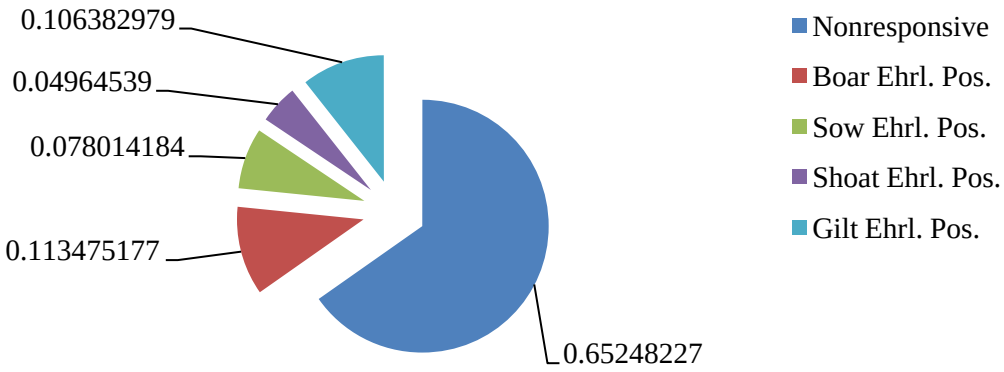


Figure 4A Population Seroprevalence of Feral Swine for Exposure to *Ehlichia* Broken Down by Age and Gender

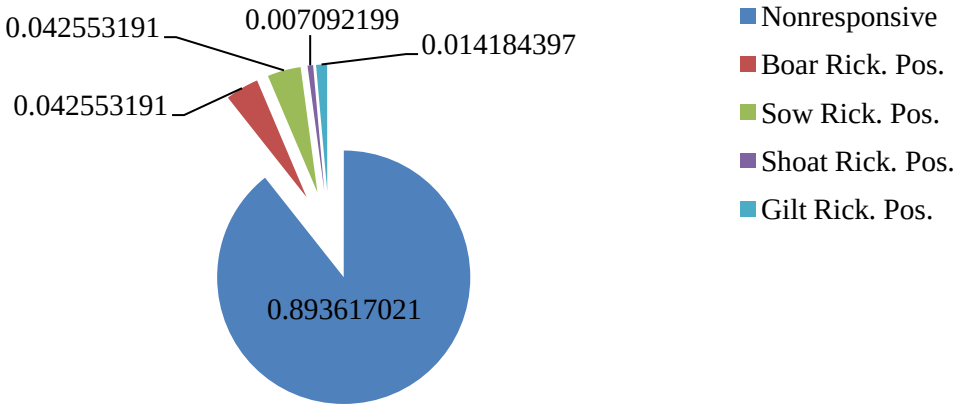


Figure 4B Population Seroprevalence of Feral Swine for Exposure to *Rickettsia* Broken Down by Age and Gender

Table 3	Summary of ELISA results showing number and percentage of samples positive according to the ecoregion of origin			
Ecoregion	Number of pigs tested for each ecoregion	Borrelia Positive	Rickettsia Positive	Ehrlichia positive
Piney Woods	115	1	48	13
Gulf Prairies and Marshes	200	3	17	56
Post Oak Savannah	65	2	10	6
BlackLand Prairie	113	4	38	3
Cross Timbers and Prairies	69	3	27	17
South Texas Plains	105	7	50	15
Edwards Plateau	76	1	23	7
Rolling Plains	107	1	28	0
High Plains	13	0	2	0
Trans-Pecos	15	0	2	0

The Gulf Prairies and Marshes site, Welder Wildlife Refuge, lies along the border area of South Texas Plains and may be influenced by both ecoregions.

Western Blot: Nineteen samples positive for exposure to *Borrelia* by ELISA assay were further analyzed by Western Blot assay. Samples 3 and 11 were likely positive for *B. turicatae* based on the established criteria described above. Those remaining samples of 1-15 were positive to both the native GlpQ, ~ 39kd, and the rGlpQ, ~ 50kd, but were weakly responsive to the whole-cell lysate. Numbers 16-19 had absorbance readings that were just below the cutoff for positive samples for Borrelia exposure based on the ELISA criteria and are also negative by the Western Blot criteria, but their outcomes suggested exposure to an undetermined Borrelia species because of their being just below the standard for positive samples by ELISA and because of their strong reactions to both the native and recombinant GlpQ. The visualization image summarizing the nitrocellulose banding following final development of the Western Blot can be found in the Appendix (A7).

Discussion

Feral pigs are spreading across the United States at a significant pace. This is especially so in Texas where nearly half of the United States feral pig population exists. Pigs in this study were taken from as far west as the Texas and New Mexico state line, meaning that pigs are surviving and dispersing across landscapes thought to be less than favorable for feral pigs based on the poor thermoregulation of *Suidae*, especially in the absence of a constant water supply. Pig populations are not expanding based on dispersal events alone. Frequently, the pigs are aided by humans in an effort to establish herds large enough to provide year-round hunting, but these transplanting events frequently provide sufficient individuals to develop a self-sustaining population. Pigs are not only expanding their own range, but they appear to be aiding the expansion of several vector species of ticks. *Dermacentor variabilis* and *Amblyomma maculatum* are possible examples of this phenomenon. Both species were consistently recovered from feral swine over areas larger than where the ticks had been previously reported. The known exposure of feral pigs to the tick vectors helped refine the hypothesis that feral pigs may be exposed at a higher rate to tick-borne pathogens of the three genera bacteria studied here. The results of this serosurvey indicate feral pigs do appear to be exposed to all three of the tick-borne pathogens of interest and at relatively high percentages.

These data are informative about previous exposure but do not address host competency or reservoir status of feral pigs for these three pathogenic bacteria. Alternate methods would have to be developed to accurately determine the state of the infection (acute versus chronic or infectious versus convalescent or exposed and not sufficiently challenged to prompt a disease response) in the feral pigs in addition to clinical studies involving the host.

Feral pigs are expanding their range and are involved at some level with the vector and pathogen. There are a couple of possible outcomes as viewed through the public health and

research perspectives. Feral pigs, because of their similar physiology to that of humans, could possibly serve as sentinel animals for these disease particular agents in the environment. On the other hand, feral pigs could just as well serve as maintenance or amplifying host, not only of zoonoses but also of epizootic agents in addition to the arthropod vectors. Either outcome warrants long term monitoring and expanded research of feral pig populations given their continued range expansion and establishment of self-sustaining populations.

CHAPTER IV

A MULTIPLEX PCR ASSAY FOR DNA DETECTION OF TICK-BORNE PATHOGENS IN THE GENERA *BORRELIA, EHRLICHIA, AND RICKETTSIA*

Chapter I reported seven species of ticks were recovered from Texas feral swine harvested from June 2008 to June 2010, and the percentage of pigs infested with ticks was between 62% and 75%. The seven species of Ixodid ticks were *Amblyomma americanum, A. cajennense,* and *A. maculatum*; *Dermacentor albipictus, D. halli,* and *D. variabilis*; and *Ixodes scapularis*. Five of these species are known vectors for one or more of the three genera of tick-borne pathogens targeted for further study. Chapter II summarized the results of antibody detection analysis of feral pig serum from pigs harvested across Texas from 2006 to 2010. The results of the serosurvey, based on those individuals tested, indicated the percentage of Texas feral pigs being exposed to *Borrelia, Rickettsia,* and *Ehrlichia* are 2%, 28% and 13%, respectively. However, the species specific exposure is unknown as is the stage or state of the exposure e.g. acute or chronic, infectious or convalescent, or exposed and not infected.

The species specific issue could begin to be resolved using PCR techniques. It is possible to determine the Genus of the pathogen using PCR methods on whole blood samples taken from feral swine. Targeting the Genus level and not a specific species at this point allows for detection of a wider range of DNA from pathogenic forms of each genus. Samples positive for a genus could be sequenced for species specific DNA determination.

A technique requiring greater trial and error would be the use of PCR in a multiplex arrangement. However, multiplex PCR would allow a sample to be tested for multiple organisms at the cost and effort of testing the same sample for a single organism once optimized. Multiplex assays can be further simplified by using either a single forward primer and multiple

reverse primers or a single reverse with multiple forward primers. The assay developed here used as single forward and three reverse primers specific to *Rickettsia, Borrelia* or *Ehrlichia*.

The development of PCR assays as a diagnostic tool has many issues outside of the actual PCR protocol itself. In this case, one of the earliest issues came out of the specific biology of each pathogens and whether the genera could be detected in a sample of whole blood. *Borrelia* spirochetes are corkscrew shaped flagellated highly motile eubacteria of the family Spirochaetaceae, and *Borrelia* are typically detected in the blood stream of mammalian hosts. Blood sampling is commonly used by researchers at the Rocky Mountain Laboratories when taking field samples (Porcella et al. 2000). So, *Borrelia* should be harvestable from whole blood samples. *Ehrlichia chaffeensis* is an intracellular obligate, immotile, Gram-negative bacteria species that displays tropism towards reticuloendothelial cells, specifically monocytes (Dawson et al. 2005). This implies that *Ehrlichia* should also be harvestable from whole blood sampling. *Rickettsiae*, like *Ehrlichia* species, are rod shaped Gram-negative intracellular obligate bacteria. However, *Rickettsia rickettsii* exhibit tropism for endothelial cells which line the blood vessels in mammalian hosts (Macaluso and Azad 2005). The assumption could be made that *Rickettsia* could be harvested from a whole blood sample. Extraction, amplification and separation of the DNA should be straightforward from this point with materials and protocols for each step being available through commercial vendors.

Materials and Methods

Pathogen DNA Detection by PCR

Primer Selection: Primers for PCR analysis were selected from the 16s gene. Primers are based on consensus sequences selected for each genus using the Ribosomal Database Project (Cole et al. 2007, Cole et al. 2009) and were selected for multiplex PCR assay using BioEdit (Hall 2009) and Primer3 (Rozen and Skaletsky 2000). A single master forward primer was used

in conjunction with three pathogen specific reverse primers. Primer sequences and relevant data are shown in Table 4.

Table 4 PCR Primers for Pathogen DNA Detection

Pathogenic Genus	Primer	Product Size	Melting Temp C°
Master Forward	5' CTCCTACGGGAGGCAGCA 3'	N/A	66.6
Rickettsia	5' TCTTATAGTTCCCGGCATTACCC 3'	794 bp	65.6
Ehrlichia	5' CCTCAGTGTCAGTATCGAACCAG 3'	397 bp	64.7
Borrelia	5' GAGTTTCACTCTTGCGAGCATAC 3'	570 bp	63.9

PCR – DNA Extraction: Blood samples were processed for DNA extraction using DNeasy® Blood &Tissue individual spin column kits and the DNeasy® Blood &Tissue Handbook protocol for Gram-negative bacteria (Qiagen, Valencia, CA).

PCR – Reaction and Conditions: Amplification was accomplished using Qiagen's Multiplex PCR kit but also attempted using Promega's GoTaq Green (Madison,WI). Twenty-five µL reactions were processed using an Eppendorf Mastercycler Personal. Reactions were comprised of 12.5 µL of Qiagen Multiplex master mix or GoTaq Green master mix, 2.0 µL of a single master forward, 1.0 µL of each genus specific reverse, 2.5 µL of Q solution for hot start reactions only (supplied with Qiagen Multiplex PCR kit), 5.0 µL of template material, and 2.0 µL for hot start reactions or 4.5 µL of Nuclease Free Water (in kit). This kit is specifically designed for multiplex PCR reactions that require hot start conditions. The cycle protocol times

and temperatures for the hot start reactions were a 95°C for 15 minutes activation step followed by 40 cycles of 94°C for 30 seconds, 62°C for 90 seconds, 72°C for 90 seconds, and 72°C for the final extension step of 10 minutes. The conditions for the standard reactions were 94°C for 2 minutes for the activation step followed by 40 cycles of 94°C for 30 seconds, 63°C for 60 seconds, 72°C for 60 seconds, and 72°C for seven minutes in the final extension step. Product was visualized in 2% agarose gel using Fotodyne's Foto/Analyst® Investigator/Eclipse Systems. The selected primer set was tested by 2-fold serial dilution to 2^{-7} in order to estimate the limit of detection for DNA available in a sample of extracted material.

Feral Pig and Sample Collections

Whole blood samples from pigs (N=233) were tested under the hot start multiplex PCR conditions described above. Samples tested were taken from eight Texas counties; Aransas, Bexar, Brazos, Caldwell, Harris, Leon, Liberty and San Patricio counties. Feral pigs were harvested in accordance with Texas A&M University IACUC Animal Use Protocol #2008-131 guidelines. Collection procedures for blood samples have been previously described.

Results

Four sets of primers were assessed against known samples under varying reaction conditions, ultimately hot start and standard conditions. Figure 5 (A and B) shows the results of four different primer sets tested under standard, image A, and hot start, image B, conditions. The primer set shown in Table 1 was selected based on no cross reactivity, absence of additional unpredicted bands and correct band sizes when tested in multiplex format against single pathogen and triple pathogen positive controls using the hot start protocol. The selected primer set was tested at 2-fold serial dilution to the 2^{-7} level and pathogens were detectable at this level.

Two of the 233 field samples, both from Welder Wildlife Refuge, were positive for the presence of *Borrelia* DNA (Figure 6). *Rickettsia* or *Ehrlichia* DNA was not detected in any of the field samples using the protocols described.

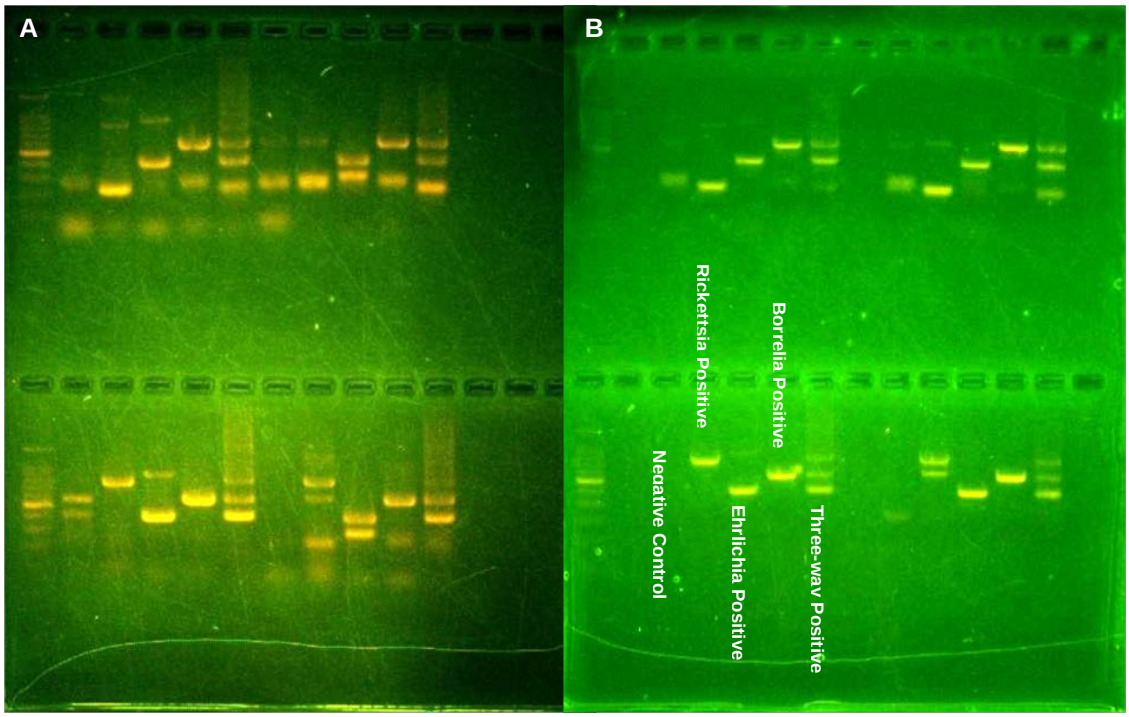


Figure 5 Image A is of an electrophoretic gel showing initial four primer sets ran in the absence of hot start conditions and separated in a 2% agarose gel. Lane assignments are as described for 5B. The primer set with labels in image B was selected for use with field samples. The other three primer sets shown followed the same layout with regard to lane assignments for both gels pictured below.

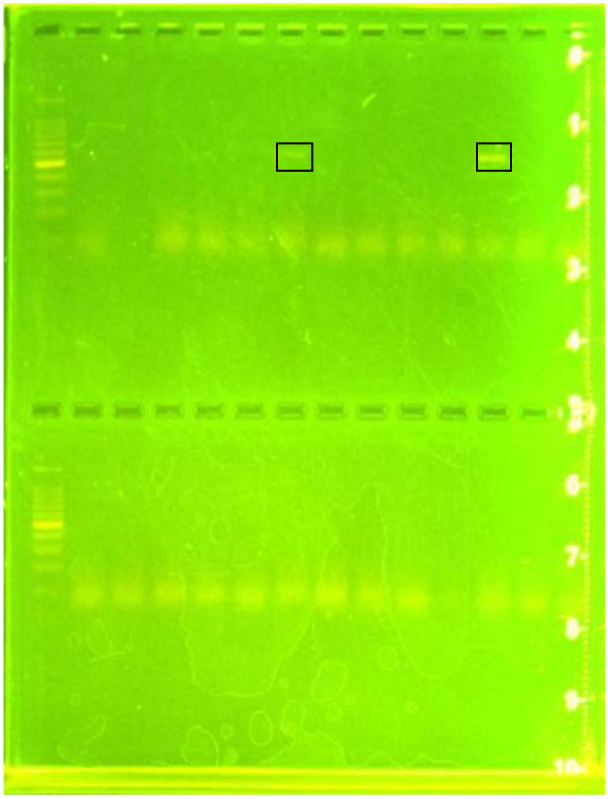


Figure 6 Image of an electrophoretic gel visualizing the outcome using the described multiplex PCR assay with two sample positive for the genus *Borrelia*.

Discussion

Serological data from Welder Wildlife Refuge show two feral pigs were positive for exposure to *Borrelia*. These were not the same two pigs PCR positive for *Borrelia* DNA. This puts the percent of pigs positive for the presence of *Borrelia* DNA and positive for previous exposure at the same level.

The disturbing factor was the absence of DNA from *Ehrlichia* and *Rickettsia* as the seropositivity was higher for these two genera. There are a number of issues that could have given false negatives. The two most likely in this case are the method of sampling the pigs and

the extraction protocol used on the whole blood samples. The extraction protocol described is for use with cell cultured bacteria and theoretically should have worked even though both *Rickettsia* and *Ehrlichia* are intracellular obligates. It is possible that this did affect the extraction process by creating additional physical barriers to DNA extraction of intracellular bacteria. The situation for *Ehrlichia* and *Rickettsia* could have further been complicated by the sampling method employed for feral swine. It is not unusual for *Borrelia* to be detected in the blood stream of mammals collected in the field using cardiac puncture. However, extraction methods are frequently more aggressive for *Ehrlichia* and *Rickettsia* than would be necessary for an organism that is free-living within the bloodstream, such as *Borrelia*. Schriefer et al. (1994) would be an example where the blood sample is incubated for four hours as compared to the 1.5 hour incubation period used with the Qiagen DNeasy® Blood &Tissue kits. It is possible that the field sampling techniques need to be altered to include tissue sampling, e.g. lymphoid tissue, for proper detection of these two organisms. The optimization, with regard to everything from collecting samples in the field to proper extraction and amplification protocols, of this type of test for feral swine may prove very beneficial in the future as feral swine continue to expand their range across North America. With their similar physiology to that of humans, feral swine could serve as a sentinel species for zoonotic agents.

CHAPTER V

CONCLUSIONS

Plainly, there is convergence on the Texas landscape of competent vectors, pathogenic bacteria, and a host for the vector. It cannot be stated at this time what the specific role of feral swine is in the natural transmission cycles (e.g. reservoir, dead-end host, amplifying host). However, feral pigs in Texas are being utilized by five tick species known to be competent vectors for a pathogenic *Rickettsia, Ehrlichia* or *Borrelia* species. The serological results of this work confirm that Texas feral swine are being exposed to these genera of bacteria. This meets the scenario needed in a vector-borne cycle for simple pathogen transmission.

Pigs and ticks are interacting at a significant level and across a large area of Texas. Tick infestations occur in all classes, gender by age, and multiple life stages of several tick species will feed on feral swine. This is especially alarming when considering 1-host ticks such as species of the genus *Boophilus*. A dispersing sub-adult feral pig infested with *Boophilus* immature ticks could potentially move the tick species a significant distance between their combined lifetimes. Deck at al. (2006) showed that pastured livestock and feral pigs do utilize landscapes in similar manners and more so in periods of limited resource. Given that feral pigs are moving across North America with success they are just as likely moving south allowing them to come in contact with *Boophilus* ticks south of the United States and Mexico border. In the face of insufficient control options of the host there would be little to hinder their reestablishment of the ticks in the southern United States.

Assemblages of the tick species found on feral hogs do differ among ecoregions and more so comparing east Texas to west Texas. This variation in assemblages suggests that feral pigs could become a bridge or intermediate host for species of ticks that have not normally come

in contact with the reservoir host and where the endemic vector does not come in contact with naïve hosts. Feral pigs appear to be influencing the distribution of at least some species of ticks and could transport a competent vector into local foci of highly pathogenic organisms thereby increasing the probability of pathogen transmission.

The serology results of this study indicate significant exposure to the three tick-borne pathogens of interest. Pigs are mounting detectable responses to these pathogens. However, nothing can be said of the status of these exposures or whether the host develops sufficient bacteriemia or viremia to be infectious to subsequent vectors. The serological data do indicate that the percentages of pigs exposed are higher than might be expected and are influenced by local ecological conditions as could be expected due to the vector species assemblage variations.

Future studies of the role of feral swine in arthropod-borne pathogen transmission cycles should be designed to attempt to culture bacteria from tagged or marked animals repeatedly sampled. This would open the door for researchers to learn about the pathogen lifecycle in the host, host resistance, reservoir or dead-end host status of the pig, and differences that may exist in the lifecycle of the pathogen in the host contrasted to the vector. Pathogen detection techniques, such as PCR methods for DNA detection, need to be further developed and effective sampling methodological protocols devised in the event feral pigs prove to be an acceptable sentinel animal.

The claim that factors influencing pathogen transmission rates are much more complicated than simply the increase or loss of biodiversity was made in the introduction of this manuscript. What specific shifts occur in local assemblages (e.g. increases in hosts, reservoirs, vectors) better predict pathogen transmission outcomes, especially when looking at vector-borne pathogens. Arthropod-borne zoonotic cycles are complicated further by vectors with longer lifecycles and multiple life stages which actively feed on different hosts than at other life stages.

This is the case with many 3-host Ixodid tick species. Some Argasid tick species host utilization not only differs by life stage but many of these will feed multiple times per stage adding yet another layer of complexity to pathogen transmission. Adding another complicating factor are ectoparasites, such as ticks, that have long periods of host association due to feeding or molting habits enabling the host to aid the further distribution of the vector across its own natural geography. This is a possible outcome when the host reshapes local ecology in the manner that feral pigs do.

There is no arguing biodiversity affects pathogen transmission nor is there any arguing that invasive species cause shifts in local biodiversity where they become established. There is also no debate that globalization has strongly influenced biodiversity changes worldwide. The United States sits atop a precarious perch in this regard. Officials in the United States strive to prevent all forms of disease but may in fact be opening humans, wildlife, domestic stock and companion pets up to more catastrophic events. West Nile Virus, though a relatively mild as a pathogenic virus for the most part, proved just how susceptible to introduced pathogens the United States is. Not only was the veterinarian community caught short by West Nile Virus, but the virus also caught public health professionals and governments flat-footed. The United States now has one of the heartiest mammalian invasive species firmly entrenched across the country. The feral pig is the quintessential example of the "One Health" concept. While its spread is a major veterinary concern, its close physiological relationship to humans should make it a major concern to public health professionals as well. There is a great deal that needs to be answered about the establishment of feral pigs in North America. Will the presence of feral pigs cause a shift towards pathogen transmission, or will the feral pig serve as a positive addition to its nonnative range in such a way as to reduce pathogen transmission?

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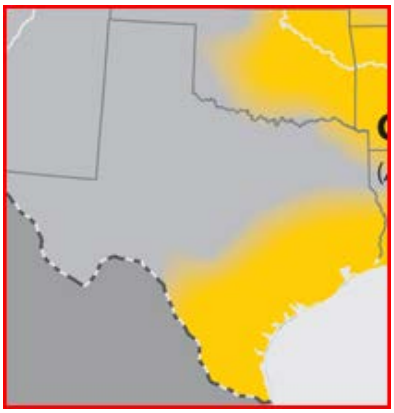
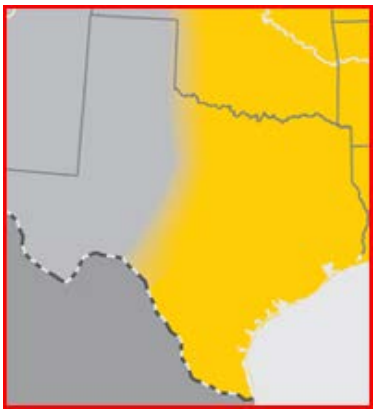
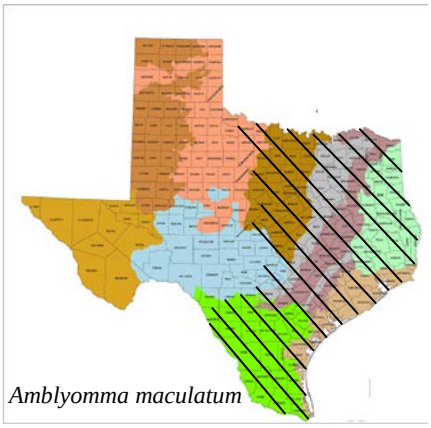
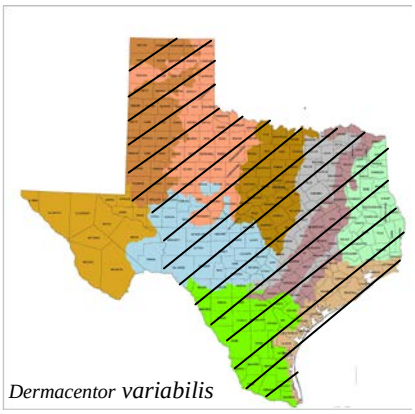
APPENDIX





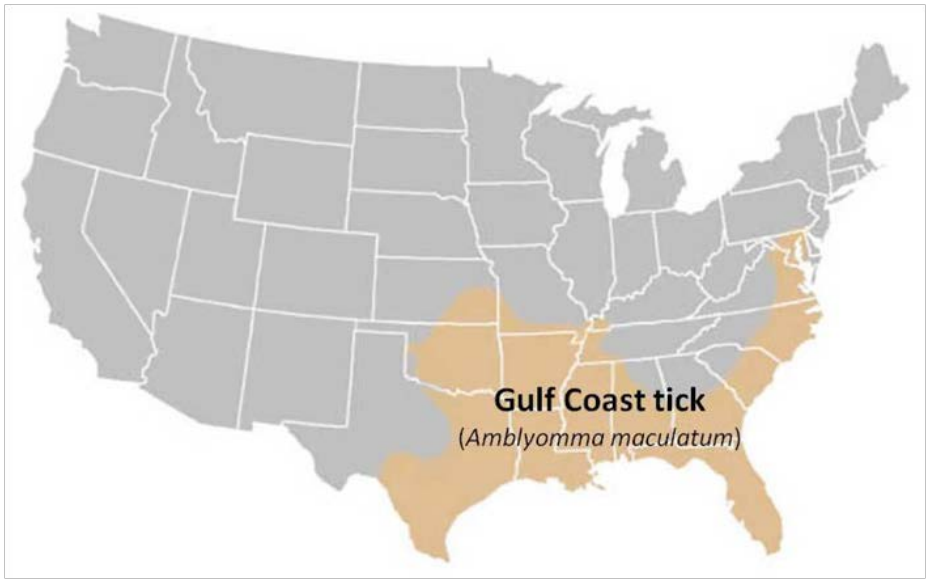


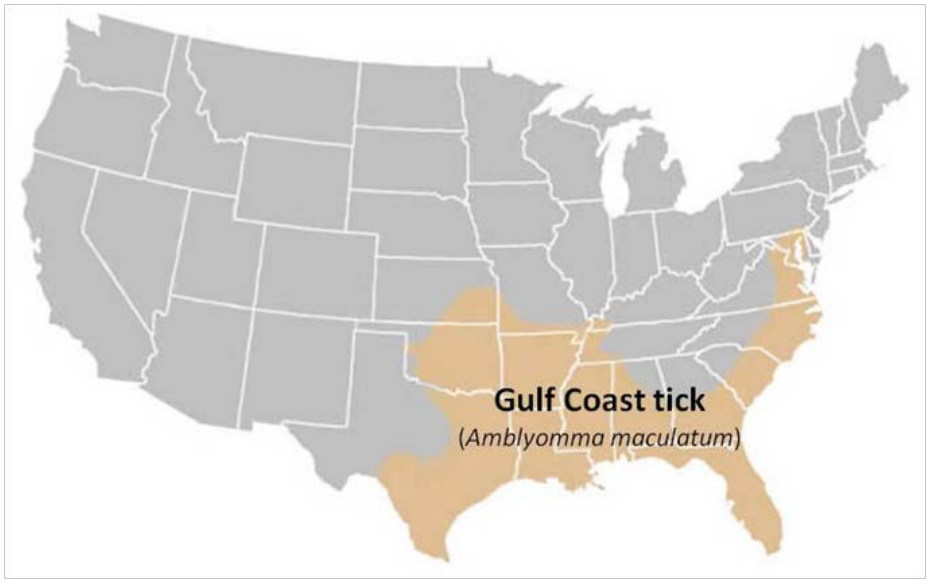


1 Piney Woods
2 Gulf Prairies and Marshes
3 Post Oak Savannah
4 BlackLand Prairie
5 Cross Timbers and Prairies

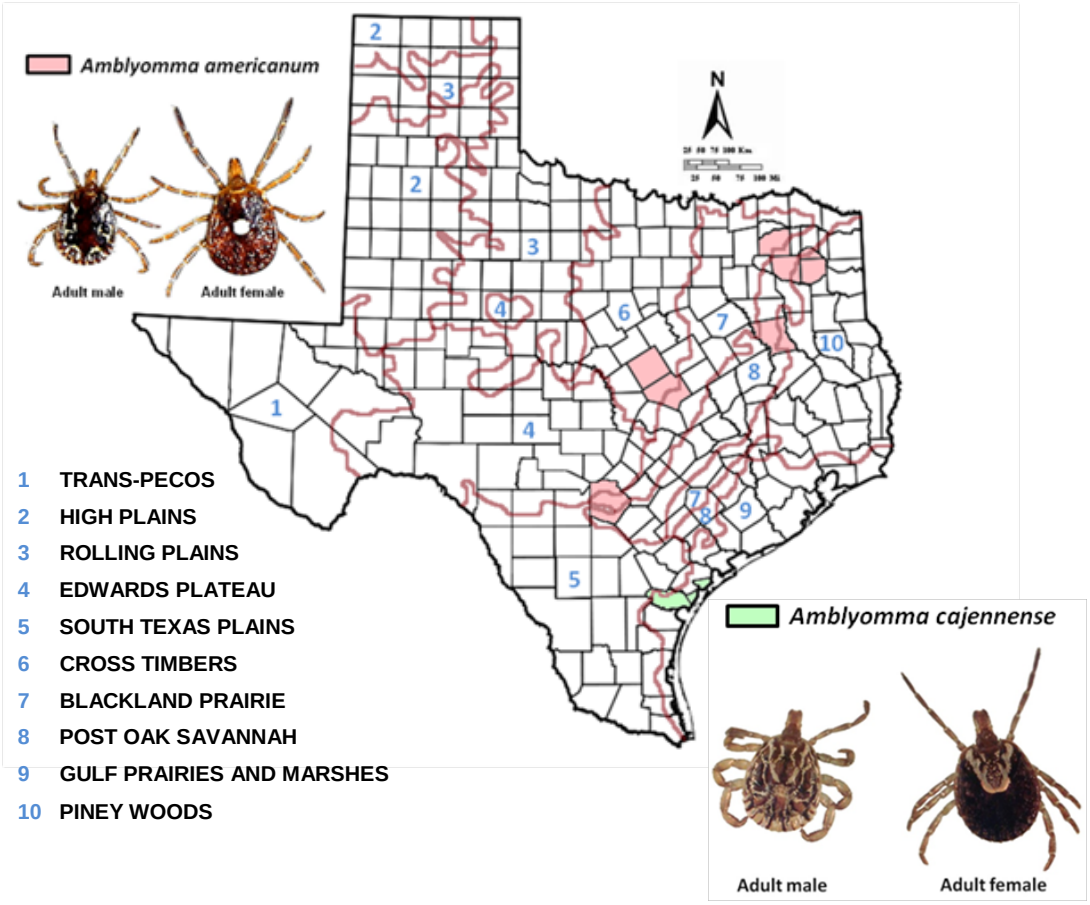
6 South Texas Plains
7 Edwards Plateau
8 Rolling Plains
9 High Plains
10 Trans-Pecos

A1 Geographical area over which *A. maculatum* and *D. variabilis* were collected from feral swine as compared to the accepted distribution areas by the Centers for Disease Control based on their published distribution maps.
Maps derived from Texas Parks and Wildlife downloadable maps at
http://www.tpwd.state.tx.us/landwater/land/maps/gis/map_downloads/
and from the Centers for Disease Control's tick distribution maps at
(http://www.cdc.gov/ticks/geographic_distribution.html)

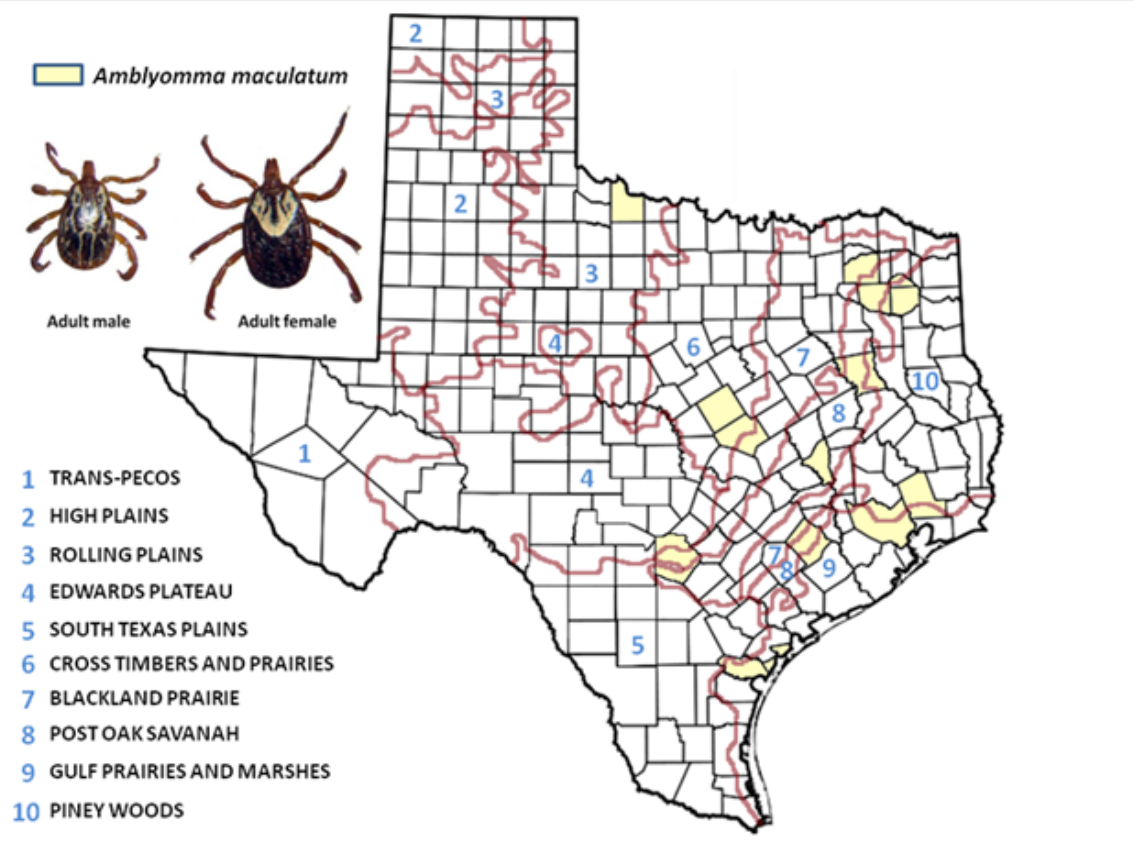




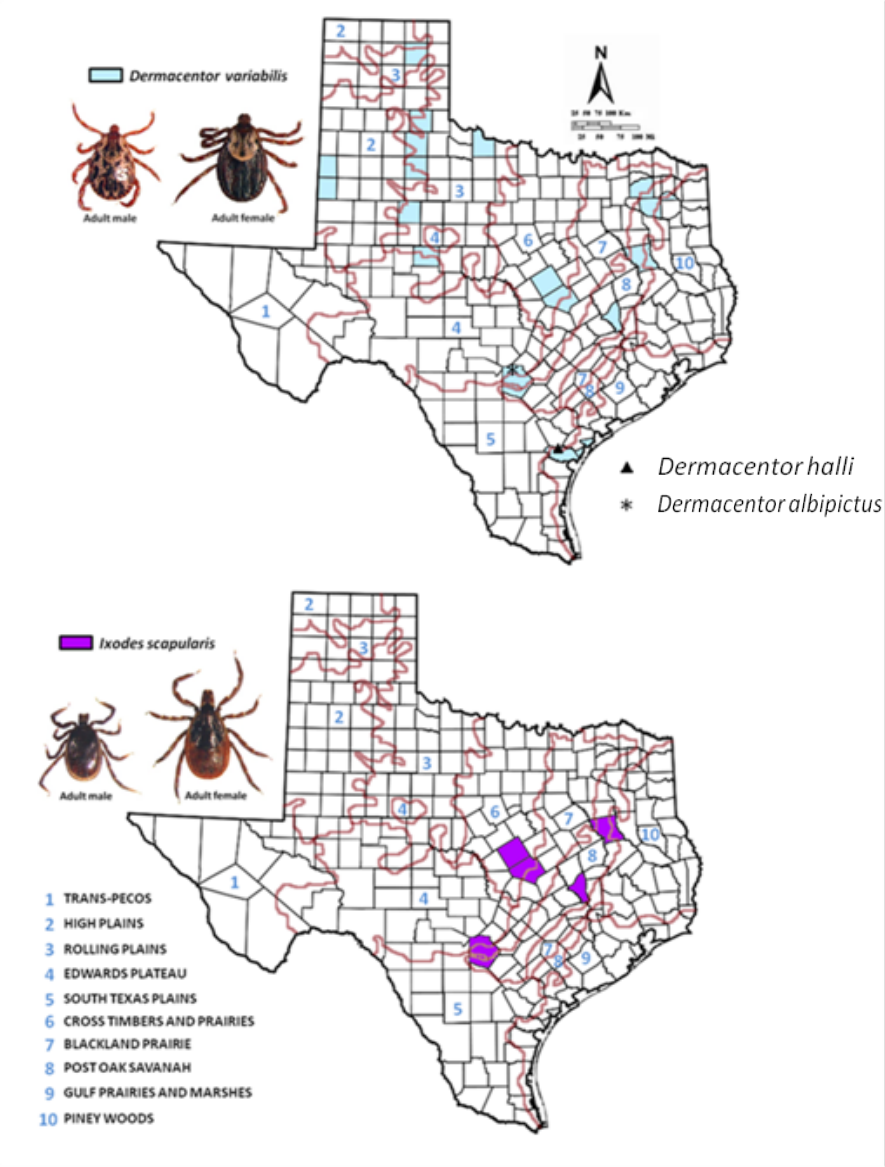
A2 Tick distribution maps for the US.
Teel, P.D. and J. Hurley. 2010. Identification and Management Guide for Ticks of the Southern Region. USDA/CSREES IPM Enhancement Grants Part 1 - Regulatory Information Network, IPM Documents and Working Groups, 8 pp.
Above: Dermacentor variabilis
Below: Amblyomma maculatum



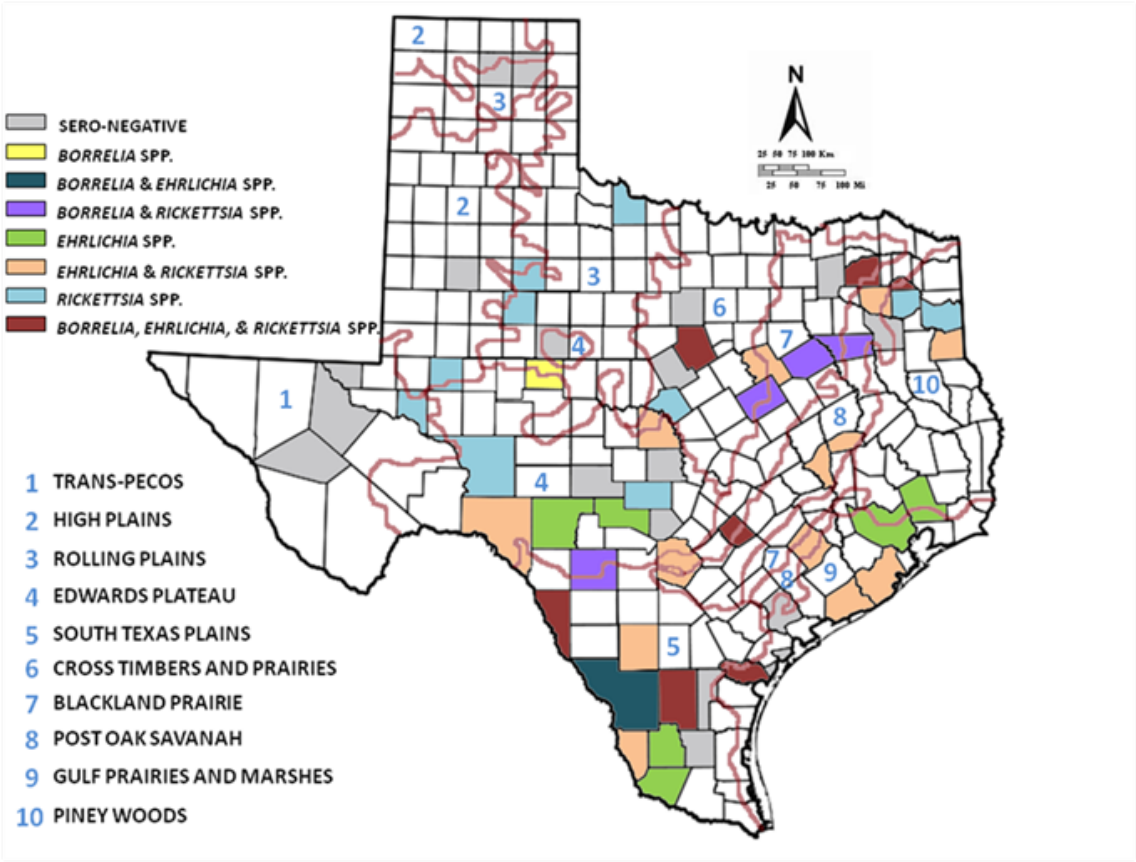
A3 Texas ecoregion map with counties shaded according to the *Amblyomma* species collected there.



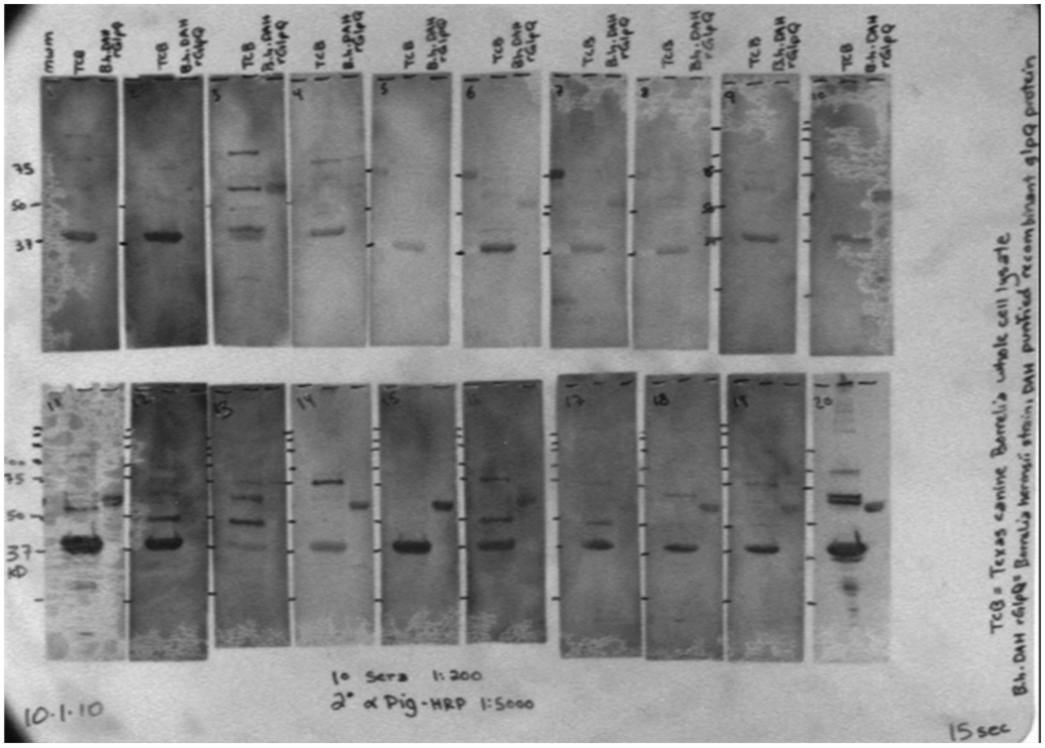
A4 Texas ecoregion map with counties shaded where *Amblyomma maculatum* was collected.



A5 Texas ecoregion maps with counties shaded according to *Dermacentor* (top) or *Ixodes scapularis* (bottom) was collected from feral swine.



A6 Texas ecoregion map with counties shaded according to the tick-borne bacteria feral swine were positive for based on serological results.



A7 Image is of nitrocellulose membranes following Western blot analysis. For all membranes lane one is the ladder discussed previously, lane two is *B. turicatae* lysate from a Texas canine cases (TCB), and lane three rGlpQ from *B. hermsii*. Membrane 20 is the positive control. Native GlpQ occurs at or near the 37kd marker and rGlpQ slightly larger than the 50kd marker.

VITA

Name and Contact Information:	David M. Sanders, Capt, USAF, PhD. Dsander8@tamu.edu Office: (979) 845 – 9494 Cell: (210) 275 – 8351
Mailing Address:	Texas A&M University Department of Entomology TAMU 2475 College Station, TX 77843-2475

Education:

B.S., Biology, The University of Memphis, 2002
M.S., Entomology, The University of Tennessee, 2005
Ph.D., Entomology, Texas A&M University, 2011